Acta

OTO-LARYNGOLOGICA

VOL 85 · JANUARY–FEBRUARY 1978 · No 1–2

EDITOR. C.-A HAMBERGER STOCKHOLM

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Conferences and Meetings

1978, Jan 16–20 The 13th Annual Otologic Surgery Course will be held at the Ear Research Institute in Los Angeles Addr F. H Linthcium, Jr , M D , Ear Research Institute, 256 South Lake Street, Los Angeles, Calif 90057, USA

1978, Jan 30–Febr 2 ARO Midwinter Research Meeting to be held at the Association for Research in Otolaryngology, Happy Dolphin Inn, St Petersburg Beach, Florida, USA. Pres David J Lim, M D , 4331 University Hospitals Clinic, 456 Clinic Drive, Columbus, Ohio 43210, USA

1978, Febr 5–11 First International Conference of the Politzer Society Inc on Tympanoplasty, Art and Science Review of 25 Years Davos/Switzerland Addr Dr Claus Jansen Winterbeckestr 11, 527 Gummersbach 1, West Germany

1978, Febr 28–March 3 A Seminar on Diagnosis and Management of Acoustic Neuromas and Skull Base Tumors will be held at the Ear Research Institute in Los Angeles Addr William F House, M D , Ear Research Institute, 256 South Lake Street Los Angeles, Calif 90057, USA.

1978, March 8–11 The 2nd International Symposium on Pediatric Otorhinolaryngology will be held in Kansas City Addr B Jazbi M D , Section of Otorhinolaryngology, The Children's Mercy Hospital, 24th at Gillham Road, Kansas City, Miss 64108 USA

1978 April 2–8 Post Graduate Course in Ear Surgery, the Dept of Otolaryngo- [illegible]

Otolaryngology, Akademiska sjukhuset, S-750 14 Uppsala, Sweden

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ACTA OTO-LARYNGOLOGICA 85 1-6 1978 KAROLINSKA SJUKHUSET FACK S-104 01 STOCKHOLM 60

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Brown, A 1953 Obliterative frontal sinusitis *Ann Otol 62*, 377.

— 1954 *Arthritis and Rheumatoid Conditions* John Wiley, New York

Brown A & Smith, B 1956 On the chemistry of the endolymph *Acta Otolaryngol* (Stockh) *46*, 408

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For more than twenty years, Marianne Coyet has served Acta Oto Laryngologica in the capacity of Editorial Secretary, but has now made up her mind to retire, with effect from January 1, 1978.

Under Frenckner's editorship, as well as during my own time, Marianne has attended in a most creditable way to the smooth running of Acta Oto Laryngologica. She has devoted considerable personal interest to our journal and has kept up the tradition of our founder, Gunnar Holmgren, of endeavouring always to maintain the highest professional standards attainable. In the course of time, Marianne has become great friends with a large number of scientists working within our sphere of interest—something that has facilitated our exceedingly good working relationship with authors and readers.

As the Editor of Acta, I feel it my pleasurable duty to convey publicly our acknowledgement of and hearty thanks to a most loyal and invaluable colleague.

Carl-Axel Hamberger

Acta Otolaryngol 85 1–9, 1978

VOLUME DISPLACEMENT OF THE TYMPANIC MEMBRANE AT STAPEDIUS REFLEX ACTIVITY IN DIFFERENT POSTURES

Studies on Variations in Perilymphatic Pressure

M Casselbrant, S Ingelstedt and A Ivarsson

From the ENT Department Malmo General Hospital University of Lund Malmo Sweden

(Received September 5 1976)

Abstract With the microflow method the volume displacement of the tympanic membrane and its direction of movement can be recorded at stapedius reflex contraction There is an outward or inward movement of the tympanic membrane which is affected by changes in posture The results indicate that the perilymphatic pressure in man varies with the posture and that these variations can be measured indirectly outside the tympanic membrane This can only be done however on condition that there are no pressure variations across the tympanic membrane This method opens up new ways of studying possible pressure changes in the inner ear in acute diseases

The intention of the present authors was to investigate possible variations in the perilymphatic pressure in man and to find out if such variations can be measured outside the tympanic membrane This investigation seemed to be of particular interest since it enabled us to observe whether the perilymphatic pressure varies with the symptoms of different types of acute sensorineural hearing loss, such as mb Meniere

In an experimental investigation on human temporal bone, Densert et al (1977) showed that the volume displacement of the tympanic membrane at standardized elicited pull on the stapedius tendon was affected by different perilymphatic pressures The results seem to indicate that any variations in the perilymphatic pressure in man can be measured by the volume displacement of the tympanic membrane during contraction of the stapedius muscle

Does the perilymphatic pressure vary with posture? Jonson & Rundcrantz (1969) showed a pressure increase in the internal jugular vein, coinciding with a change from sitting to recumbent position This increase amounted to about 10 cm H_2O, identical with the intracranial pressure increase demonstrated by Dawson (1967)

Experimental studies on animals have been performed by Ahlen (1951) and Keith & Allen (1963), who found that pressure in the cerebrospinal fluid is reflected in the perilymph and that an increase in the CSF pressure increased the perilymphatic pressure to the same extent Beentjes (1972) showed that a change in the CSF pressure affects the perilymphatic as well as the endolymphatic pressure

We assume that the pressure of the inner ear in man increases to the same extent as the venous and the CSF pressure when the posture is changed from sitting to recumbent In

These results were reported at Lakarsallskapets Riksstamma Stockholm 1975

This study was supported by the Swedish Medical Research Council (no B77 17X-04981-01) and the Swedish National Defence Research Institute (proj no 506 H352)

vestigations on humans have been performed by Corso (1962), Miltich (1968) and Macrae (1972), who made comparative determinations of the hearing threshold at changes in posture and were able to show a lowering of the hearing threshold in inverted body position According to Miltich and Macrae this may be caused by increased intralabyrinthine pressure Macrae (1972) also studied the change in acoustic impedance with change in posture, and Klockhoff et al (1966) showed similar impedance changes on compression of the neck veins None of these authors have taken into account the change in middle ear pressure which occurs when the posture is changed or when the neck veins are compressed This may affect the results

Andreasson et al (1976) studied the effect on the middle ear mucosa of vein pressure increase, which occurs when the posture is changed from sitting to recumbent They found that the increased congestion of the middle ear mucosa (about 10 μl) in the recumbent position causes an increase in the middle ear pressure amounting to +1 cm H_2O, when compared with the sitting position with intact tympanic membrane and closed Eusta an tube With the impedance method, in ing measurements with a closed auditory l, the variations of the congestion in the ympanic membrane and the skin in the auditory canal may influence the results when the body position is changed

In an earlier investigation the authors Casselbrant et al (1977) have presented an open microflow method for quantitative recording of the volume displacement of the tympanic membrane upon stapedius reflex contraction The intention was also to see what middle ear variables influence the stapedius reflex response in a sitting position

The aims of the present investigation were to find out

1) if possible shifts in the hearing threshold due to changes in posture are caused by changes in the middle ear pressure

2) if the stapedius reflex response is influenced by changes in posture at the san middle ear pressure,

3) if the change, in the perilymphatic pre sure at different postures can be measured ou side the tympanic membrane

METHODS

Measuring of the hearing threshold

A Békesy audiometer (E800 Grason Stadl was used to determine the hearing thresho by an interrupted fixed frequency tone of kHz during 3 min

Measuring of the middle ear pressure

An electroacoustic impedance bridge (ZO Madsen) was used, by which the middle e pressure was estimated from a tympanogra at a pressure change in the auditory canal ± 20 cm H_2O

Measuring of the stapedius reflex response

The stapedius reflex contraction was elicit by acoustic stimulation (1 kHz 105 dB rel IS 1964) and recorded in the contralateral e with an open microflow method by which t movement direction of the tympanic mer brane and its volume displacement can l recorded quantitatively (For details, see Ca selbrant et al , 1977)

Changes in posture

The investigations were made with the su ject sitting in a heart chair which could l adjusted so that the upper half of the body w in the position planes of 85° (sitting), 30° 20 10° and 0° (recumbent) S_1–S_4 means sitti position and R_1–R_2 recumbent The values a the mean values from 10 recordings ma during 3 min after the subject had equilibrat the middle ear properly during 5 min

MATERIAL

Ten subjects belonging to tubal function grou Ib the same as were used by Casselbrant et a

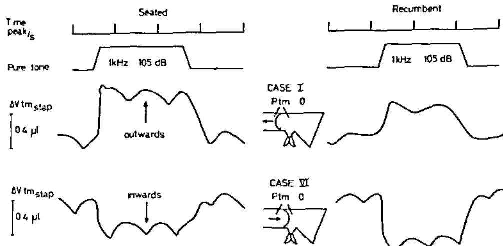


Fig. 1. Recording in two cases of outward (case I) or inward (case VI) movements of the tympanic membrane at stapedius reflex contraction in seated and recumbent positions. P_{tm}, pressure difference across the tympanic membrane.

(1977). The subjects were also able to equilibrate the middle ear to atmospheric pressure in a sitting as well as in a recumbent body position.

PERFORMANCE AND RESULTS

Determination of hearing threshold in sitting and recumbent positions

It was checked at the start that the subjects in the sitting position had atmospheric pressure in the middle ear and that they either did or did not equilibrate the middle ear pressure during investigations in the recumbent position.

There was no statistical difference ($p>0.05$) between the hearing thresholds in the sitting and the recumbent position, whether the subjects had equilibrated or not.

Comments. During the investigation of the hearing threshold at different middle ear pressures a change of 2.2 dB ($p<0.001$) was followed by a relative overpressure in the middle ear of about 2 cm H_2O as compared with the atmospheric pressure in the middle ear (Casselbrant et al., 1977).

Determination of middle ear pressure in sitting and recumbent positions

The middle ear pressure was examined in the sitting and the recumbent position with and without equilibration of the middle ear. The middle ear pressure increased, on average, by +1 cm H_2O (S.D. = 0.34, S.E. = 0.1) in the recumbent position, as compared with the sitting position when the subjects did not equilibrate.

VOLUME DISPLACEMENT OF TYMPANIC MEMBRANE AT STAPEDIUS REFLEX CONTRACTION

(*a*) *In sitting and recumbent position*

An acoustically elicited stapedius reflex contraction causes either an outward or an inward movement of the tympanic membrane in a sitting position (Casselbrant et al., 1977). When the posture is changed from the sitting to the recumbent, the stapedius reflex response pattern measured as the volume displacement of the tympanic membrane will be affected when a pure tone stimulus (1 kHz, 105 dB) is produced in the contralateral ear (Fig. 1).

Table I *The initial volume displacement of the tympanic membrane, $\Delta V_{tm_{stap}}$, in the sittir position (S_1–S_3) and in the recumbent position (R_1–R_2) at pure tone stimulus (1 kHz 105 dB ISO 1964) produced in the contralateral ear*

Middle ear pressure=P_{atm}
The initial volume displacement is the mean value from 10 recordings in each body position
+=outward movement −=inward movement of the tympanic membrane

Case	Left (probe ear)					Right (probe ear)				
	S_1	R_1	S_2	R_2	S_3	S_1	R_1	S_2	R_2	S_3
I	+0 50	+0 38	+0 53	+0 42	+0 52	+0 37	+0 28	+0 36	+0 30	+0 40
II	+0 40	+0 33	+0 39	+0 30	+0 38	+0 44	+0 46	+0 46	+0 42	+0 46
III	+0 40	+0 34	+0 37	+0 32	+0 37	No rec	No rec	No rec	No rec	No rec
IV	+0 10	−0 08	+0 12	−0 06	+0 10	+0 09	−0 04	+0 05	−0 04	+0 04
V	+0 06	−0 11	+0 06	−0 09	+0 05	+0 20	−0 08	+0 21	−0 09	+0 23
VI	−0 32	−0.53	−0 29	−0 49	−0 36	−0 38	−0 54	−0 32	−0 48	−0 35
VII	+0 10	0	+0 10	+0 03	+0 13	−0 14	−0 21	−0 15	−0 23	−0 18
VIII	+0 11	0	+0 11	0	+0 13	+0 04	−0 06	+0 04	−0 04	+0 03
IX	+0 54	+0 54	+0 54	+0 54	+0 53	+0 30	+0 14	+0 30	+0 17	+0 27
X	−0 06	−0 20	−0 07	−0 20	−0 07	−0 09	−0 16	−0 12	−0 17	−0 11

Table I gives the initial volume displacement of the tympanic membrane at stapedius reflex contraction (cf initial volume displacement, Casselbrant et al, 1977) The values are the mean values from 10 recordings in each body position The subjects had equilibrated the middle ear pressure to atmospheric … after each change of posture

… ubjects with great outward movement of …e tympanic membrane in the sitting position show a smaller outward movement in the recumbent position (cases I, II, III and IV bilateral, see Table I) In all cases except two (cases II right and IX left), there are statistically significant changes ($p<0\ 001$)

Subjects whose tympanic membranes move inwards in the sitting position get a stronger inward movement in the recumbent position (cases VI X bilateral and VII right, see Table I) The change is statistically significant for these ears ($p<0\ 001$)

Subjects with a slight outward movement … the sitting position show a change in the mov… ment direction of the tympanic membrane … an inward movement in the recumbent po… tion (cases IV, V bilateral and VIII right, s… Table I)

(b) Gradual change in posture

The stapedius reflex response in differe… postures has also been studied in 2 subjec… (cases V and VI) with the upper half of t… body in the positions of 85° (sitting), 30°, 2… 10° and 0° (recumbent), respectively Imm… diately after each change in posture the su… jects equilibrated the middle ear pressure … ambient pressure Table II shows the diffe…

Table II *The initial volume displacement of the tympanic membrane at stapedius reflex co… traction $\Delta V_{tm_{stap}}$ at gradual change in posture*

The values are the mean values from 10 recordings in each posture
+=outward movement −=inward movement at 1 kHz 105 dB rel ISO 1964

Case	Ear	S	20°	S_2	10°	S_3	0°	S_4	30°
V	Left	+0 05	0 03	+0 04	0 07	+0 05	−0 11	+0 06	+0 03
	Right	+0 23	0 10	+0 18	+0 04	+0 20	−0 07	+0 16	+0 12
VI	Left	−0 33	0 35	0 32	−0 41	−0 33	−0 48	−0 32	No rec
	Right	−0 36	0 38	−0 35	−0 40	0 37	−0 52	−0 38	No rec

Table III *Double examinations of the initial volume displacement of the tympanic membrane, ($\Delta V_{tm_{stap}}$), in the sitting (S_1–S_3) and the recumbent (R_1–R_2) position at pure tone stimulus (1 kHz, 105 dB rel ISO 1964) produced in the contralateral ear*

The middle ear pressure – P_{atm}
The initial volume displacement is the mean value from 10 recordings in each posture

Case	Exami nation	Left S_1	Left R_1	Left S_2	Left R_2	Left S_3	Right S_1	Right R_1	Right S_2	Right R_2	Right S_3
I	1st	+0 50	+0 38	+0 53	+0 42	+0 52	+0 37	+0 28	+0 36	+0 30	+0 40
	2nd	+0 50	+0 31	+0 51	+0 42	+0 51	+0 36	+0 30	+0 39	+0 31	+0 38
V	1st	+0 06	−0 11	+0 06	−0 09	+0 05	+0 20	−0 08	+0 21	−0 09	+0 23
	2nd	+0 10	−0 07	+0 08	−0 08	+0 07	+0 15	−0 06	+0 14	−0 04	+0 13
VI	1st	−0 32	−0 53	−0 29	−0 49	−0 36	−0 38	−0 54	−0 32	−0 48	−0 35
	2nd	−0 34	−0 50	−0 33	−0 48	−0 32	−0 41	−0 45	−0 35	−0 46	−0 36

ence in the "response" amplitude recorded at 0°, 10°, 20° and 30° body angle in relation to response amplitude in the sitting position (S_1, S_2, S_3 and S_4) The essential change in the stapedius reflex response occurs at 10° body angle

(*c*) *Time factor after change in posture*

In case V the influence of the time factor on the stapedius reflex response was studied after a change from the sitting to the recumbent position The essential change of the stapedius reflex response occurred immediately

(*d*) *Reproducibility of stapedius reflex response*

As an illustration of the reproducibility of the volume displacement of the tympanic membrane at stapedius reflex contraction in different postures, Table III presents results of re examinations of 3 subjects (5 ears) In these 3 subjects we found good agreement between the recorded volume displacements of the tympanic membrane during the different examinations These took place at intervals of several months

INFLUENCE OF STAPEDIUS REFLEX CONTRACTION ON MOVEMENT DIRECTION OF TYMPANIC MEMBRANE

A comparison of the results of the volume displacement of the tympanic membrane and its movement direction at stapedius reflex contraction in different postures (Table I) and at different middle ear pressures (Casselbrant et al , 1977) shows that the material can be divided into four groups

Group I an outward movement of the tympanic membrane in the sitting and the recumbent position and at different middle ear pressures

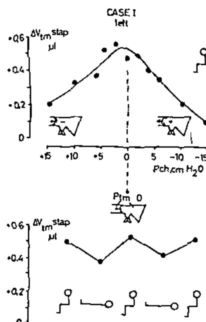


Fig 2 Volume displacement of the tympanic membrane at stapedius reflex contraction ($\Delta V_{tm_{stap}}$) at different middle ear pressures (upper curve) produced in a pressure chamber (P_{ch}) in the sitting position and in the sitting –recumbent position (lower curve) with atmospheric pressure in the middle ear i e group I

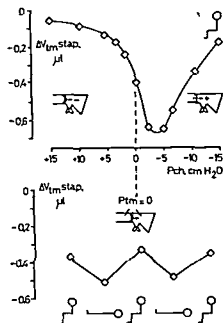


Fig 3 Group II

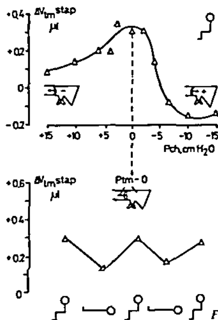


Fig 4 Group III

sures Example of recording in Fig 2 The material consisted of 5 ears (3 subjects)

Group II an inward movement of the tympanic membrane in the sitting and recumbent position and at the different middle ear pressures Example of recording in Fig 3 The material consisted of 5 ears (3 subjects)

Group III an outward movement of the tympanic membrane in the sitting and the recumbent position There is a change of movement direction at relative overpressure, i e when the tympanic membrane is outside its neutral position (Fig 4) The material consisted of 4 ears (3 subjects)

Group IV an outward movement of the tympanic membrane in the sitting position When the tympanic membrane is outside its neutral position (relative overpressure in the middle ear) and in the recumbent position there is a change of the movement direction to an inward movement (Fig 5) The material consisted of 5 ears (3 subjects)

DISCUSSION

A comparison between the sitting and the recumbent position shows a change in stapedius reflex response, recorded as the volume displacement of the tympanic membrane at identical middle ear pressures In some cases there is also a change in its movement direction at an acoustically elicited stapedius reflex contraction (Fig 5) The stapedius reflex response patterns, recorded outside the tympanic membrane, are identical with those found by Densert et al (1977) in results from

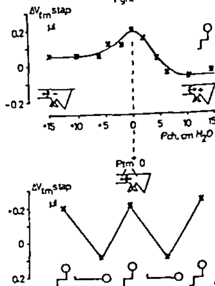


Fig 5 Group IV

experimental investigations on human temporal bones These authors showed that the volume displacement of the tympanic membrane at artificial stapedius reflex contraction was affected by different perilymphatic pressures At increased perilymphatic pressure they recorded a gradual decrease in the volume displacement of the tympanic membrane when the movement direction of the tympanic membrane was outward They recorded the reflex response upon momentarily loading (1 3 g) the tendon of the M stapedius They also found that one temporal bone showed a change in the movement direction of the tympanic membrane from outward to inward movement at an increased perilymphatic pressure

The agreement between the present results as regards the volume displacement of the tympanic membrane at stapedius reflex contraction and the model experiments on human temporal bones by Densert et al (1977) indicates that the perilymphatic pressure increases with a change in body position from sitting to recumbent This may be assumed to be due to an increase in the pressure in the internal jugular vein (Jonson & Rundcrantz 1969) and of the CSF pressure (Dawson 1967) Another parallel with the internal jugular vein pressure appears from the finding of Jonson & Rundcrantz (1969) i e that the essential pressure increase occurs at the change in posture from 20° to 0° (horizontal plane) This agrees with the results of our investigation showing the change in the volume displacement at stapedius reflex contraction following change in posture (Table II) The greatest change in the volume displacement of the tympanic membrane at stapedius reflex contraction occurs near the horizontal plane (10°–horizontal plane) It is thus improbable that the difference in the volume displacement of the tympanic membrane at stapedius reflex contraction is caused by the changed gravitational force exerted on the ossicular chain upon change of posture

When it comes to determining possible variations in the perilymphatic pressure by recording the volume displacement of the tympanic membrane at stapedius reflex contraction there is a risk of error As has been shown (Casselbrant et al 1977) the stapedius reflex response and hearing threshold are affected by the middle ear pressure At a change from the sitting to the recumbent position there is a pressure increase of on an average 1 cm H_2O due to the increased congestion in the middle ear mucosa if the middle ear is not equilibrated after the change in posture

It should be observed that as little as 1 cm H_2O relative overpressure in the middle ear can markedly affect the stapedius reflex response (Fig 3) A relative overpressure of 1 cm H_2O in the middle ear does not affect the hearing threshold This is in agreement with the results of Miltich (1968) and Macrae (1972)

The increase in hearing threshold and acoustic impedance which these authors found during investigations in the inverted position have mainly been ascribed to the increase in intralabyrinthine pressure However in the inverted position our investigations show a middle ear pressure increase of 3–5 cm H_2O which must be taken into account

There is another possible source of error to be avoided in impedance measuring namely the congestion in the tympanic membrane and the skin of the auditory canal at changes in posture The results of impedance measurements are dependent on the volume change of the auditory canal With our open microflow method this error can be avoided

Our results together with the results from the model experiment by Densert et al (1977) on human temporal bones show that it is possible to measure variations in the perilymphatic pressure on the outside of the tympanic membrane—provided that the middle ear pressure can be controlled This is further confirmed by the reproducibility of the stapedius reflex response between different examination occasions (Table III)

An attempt should be made to explain why

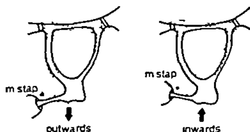


Fig 6 An explanation of the different movement directions of the tympanic membrane at stapedius reflex contraction

the tympanic membrane at stapedius reflex contraction has different opposite movement directions The reason should be sought in the anatomical relations between the stapes and the stapedius tendon At stapedius reflex contraction according to Bel et al (1976), there is a pedal movement of the stapedius foot plate in the oval window If the stapedius tendon when leaving the eminentia pyramidalis is located outside the stapedius head, a contraction of the stapedius muscle will cause an out rd movement of the tympanic membrane 6 outwards) On the other hand, if the dom leaves the eminentia inside the head of the stapes, it will cause an inward movement (Fig 6 inwards)

Why is the volume displacement of the tympanic membrane at stapedius reflex contraction affected by the middle ear pressure or body position and why do some ears show a reversal in movement direction? A decrease of the pressure in the auditory canal or an increase in the perilymphatic pressure results in pushing the stapedius foot plate out of the oval window (Densert et al 1977) In this way the relation between the head of the stapes and the stapedius tendon attachment is changed, which in its turn results in a change in the volume displacement of the tympanic membrane In some ears even the direction of the movement is reversed

After assessing the middle ear pressure according to a technique described by Elner et al (1971) it is possible by changing the surrounding pressure, to bring the tympanic membrane into its neutral position and to make measurements of the variations in the perilymphatic pressure in subjects not belonging to tubal function group Ib

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ZUSAMMENFASSUNG

Durch Messungen der Volumenschwankung im Gehörgang können mit einer Mikroströmungsmethode die Lageveränderungen sowie die Bewegungsrichtung des Trommelfells bei stapediusreflexbedingter Kontraktion registriert werden Es wurden Trommelfellbewegungen nach innen oder nach außen registriert bei denen die Korperstellung von Einfluß war Die Resultate zeigen, daß sich der perilymphatische Druck beim Menschen mit der Korperstellung andert und daß diese Druckänderungen indirekt außerhalb des Trommelfells gemessen werden konnen Dies ist allerdings nur unter der Voraussetzung moglich daß keine Druckanderungen über dem Trommelfell erfolgen Die Methode eroffnet neue Moglichkeiten für das Studium etwaiger Druckanderungen im Innenohr bei akuten Erkrankungen

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THE EFFECTS OF THE VACUUM ON VASCULAR PERMEABILITY OF THE MIDDLE EAR

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Abstract Following local application of various degrees of negative pressure to the middle ear the authors observed changes in vascular permeability in guinea pigs using Majno s vascular labelling technique Increased permeability and effusions were seen in all experimented middle ears Any pressure below −5 mm of mercury induced middle ear effusions Accordingly a decrease in pressure developed in the middle ear cavity may cause transdation of serum drawn from the submucosal vessels resulting in fluids in the middle ear of aero-otitis In such a circumstance mast cells which are easily influenced by atheric pressure may be involved in increasing vas permeability

It is a well known fact that aero-otitis media or aural barotrauma is a disease which occurs as a result of sudden change in atmospheric pressure during flying at high altitudes, working in a caisson or diving in deep waters The phenomenal growth of commercial air transport or exploratory travels in space makes these problems of special interest and importance to the general medical profession

The common symptoms of aero otitis media are characterized by pain tinnitus injection and invagination of the tympanic membrane hearing loss, effusion or hemorrhage in the middle ear cavity

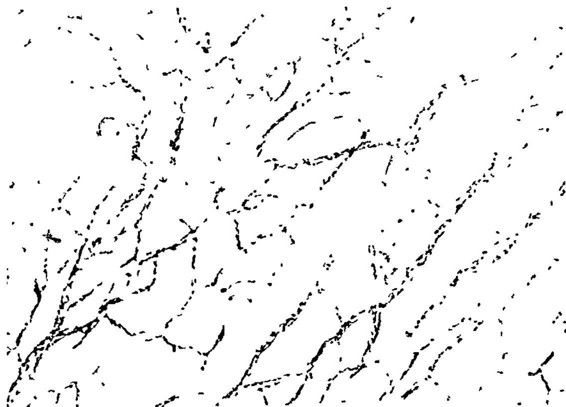
Several experimental studies have appeared in the literature to clarify the pathogenesis of the middle ear effusion in such circumstances (Aschan, 1948, Flisberg et al 1963 Paparella et al , 1970)

In our present study, we have attempted to observe the vascular change of permeability in the middle ears of guinea pigs using Majno s vascular labelling technique (Majno et al 1961) after locally applying various degrees of negative pressure to the middle ear cavity

MATERIAL AND METHOD

Seventeen healthy guinea pigs weighing 220-400 mg were used in this study The animals were anesthetized with Urethane A submandibular approach was applied to reach the tympanic bulla After exposure of the tympanic bulla, a tiny opening was made with a 2 mm round cutting bur The tip of a fine polyethylene tube was inserted It was sealed by packing gauzes containing physiological saline Subsequently, a colloidal suspension of carbon particles (200 Å) was injected into the external jugular vein (0 1 ml/100 g of body weight) Groups of experimental animals were subjected to varying degrees of negative pressure, for a fixed duration of time The negative pressure was supplied by a regular suction machine with which the negative pressure could easily be controlled to the desired value

The animals were sacrificed about 30–[illegible] minutes after the injection of carbon black when almost all carbon has been cleared from



1 Surface preparation of guinea pig middle ear mu a after applying negative pressure of −5 mmHg A hily developed arboreal pattern of blackened vessels indicates that considerable leakage of carbon has occurred (×100)

culating blood The middle ear mucosa was moved and prepared as surface specimens observation under the dissection micro-ope They were placed in 10% formalin lution for 24 hours, dehydrated in gradient luted ethanol and then placed in xylene to ake them clear Several ears were used for onventional histopathological study with ematoxylin and eosin stain Light micro-copic observation was carried out in these pecimens

RESULTS

negative pressure of −5 mmHg was applied o the middle ears of 4 guinea pigs for 5 minutes The cleared middle ear mucosa from treated guinea pigs showed a slightly de-veloped arboreal pattern of blackened vessels, indicating that considerable leakage of carbon had taken place (Fig 1) However, labelling was limited to capillaries, venules and small veins No tendency of extending to adjacent tissues was noted Arterioles and artery showed no carbon leakage throughout their walls A considerable amount of amber colored, clear, and low viscous middle ear fluid was obtained from all treated ears Their cytology showed that these effusions contained essentially no cells nor cellular materials, although occasional red blood cells were found Histologically, middle ear mucoperiosteum removed at this stage was slightly edematous, without any indication of hemorrhage Small blood vessels labelled with carbon were clearly distinguished No pathologic findings were observed in control ears

The middle ears of 4 guinea pigs were exposed for 5 minutes at the negative pressure of



2 Sectioned specimen of guınea pıg mıddle ear mu osa after applyıng negatıve pressure of −10 mmHg Vascular labellıng ıs seen ın the walls of small blood sels and subepıthelıal edema ıs noted (×200)

−10 mmHg Carbon labellıng of small vessels was more extensıve than that seen at prevıous stages, but was sımılar ın dıstrıbutıon In addıtıon, local hemorrhages were sometımes found ın the submucosa of treated mıddle ears The areas correspondıng to hemorrhages appeared

taıned from 2 out of 4 guınea pıgs Cytology revealed that 2 remaınıng ears also contaıned a consıderable amount of red blood cells However, these fluıds had no tendency to coagulate Hıstologıcally, the mucoperıosteums appeared to be edematous ın most cases (Fıg 2) Occasıonal submucosal hemorrhages were detected

For a perıod of 5 mınutes the mıddle ears of 3 guınea pıgs were subjected to a negatıve pressure of −20 mmHg of mercury Car labellıng revealed more ıncreased vasc permeabılıty ın small blood vessels

Bloody mıddle ear fluıds were obtaı from all treated ears The mucoperıoste were edematous and succulent ın all cas Local hemorrhages were noted frequently thıs stage

Three guınea pıgs were subjected, for mınutes, to a negatıve pressure of −30 mm ın the mıddle ears

A remarkable ıncrease ın vascular perm abılıty was noted ın the small vessels vacuum treated mıddle ears Heavy label was vısıble throughout their walls The extr vascular blackenıng was also noted ın m experımented anımals Bloody fluıds were o taıned ın all cases Cytology revealed th

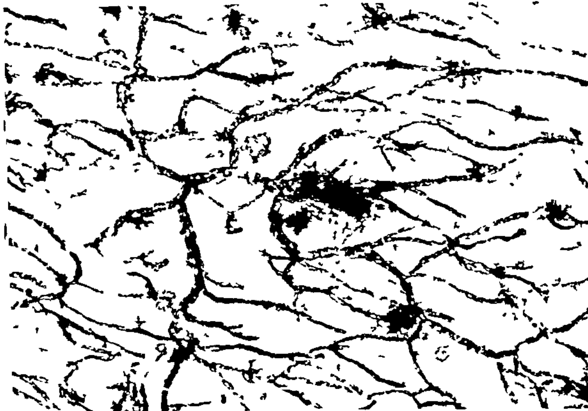


Fig 3 Surface preparation of middle ear mucosa after applying negative pressure of 50 mmHg Vascular labelling by carbon particles is clearly observed The extravascular blackening is present in the adjacent tissues of small blood vessels (×200)

most were red blood cells Histologically the mucoperiosteums showed interstitial edema but no emigration of leukocytes was noted

A negative pressure of −50 mmHg was applied to the middle ears of 3 guinea pigs for 5 minutes

The appearance of the cleared middle ear mucoperiosteums was similar to that of the animals exposed to negative pressure of −30 mmHg The degree of blackening in small vessels however was outstandingly extensive compared with any of the previous cases In addition to capillaries venules and veins and also arterioles may also be involved The carbon deposits were confined to the walls of the affected vessels Furthermore various degrees of extravascular blackening were always present in adjacent tissues of small blood vessels of experimented middle ear mucosa (Figs 3 4) It was probably caused by leakage of carbon particles through the endothelial gaps or ruptured blood vessels The fluids obtained from treated middle ear cavity were bloody Some of them tended to coagulate indicating that they might have consisted partly of blood

Histologically the mucoperiosteums showed edema in all cases The labelling of small vessels with carbon was clearly observed in sectioned specimens Hemorrhages were noted to a varying extent in the vacuum treated middle ear mucosa No perforation of the tympanic membrane was noted in any of the experimentally treated animals

DISCUSSION

Leaking blood vessels can be marked in vivo by intravenously injecting a colloidal suspen



Fig 2 Sectioned specimen of guinea pig middle ear mucosa after applying negative pressure of −10 mmHg Vascular labelling is seen in the walls of small blood vessels and subepithelial edema is noted (×200)

−10 mmHg Carbon labelling of small vessels was more extensive than that seen at previous stages, but was similar in distribution In addition, local hemorrhages were sometimes found in the submucosa of treated middle ears The areas corresponding to hemorrhages appeared black due to the extension of carbon into adjacent tissues Bloody middle ear fluids were obtained from 2 out of 4 guinea pigs Cytology revealed that 2 remaining ears also contained a considerable amount of red blood cells However, these fluids had no tendency to coagulate Histologically the mucoperiosteums appeared to be edematous in most cases (Fig 2) Occasional submucosal hemorrhages were detected

For a period of 5 minutes the middle ears of 3 guinea pigs were subjected to a negative pressure of −20 mmHg of mercury Carbon labelling revealed more increased vascular permeability in small blood vessels

Bloody middle ear fluids were obtained from all treated ears The mucoperiosteums were edematous and succulent in all cases Local hemorrhages were noted frequently at this stage

Three guinea pigs were subjected, for 5 minutes, to a negative pressure of −30 mmHg in the middle ears

A remarkable increase in vascular permeability was noted in the small vessels of vacuum treated middle ears Heavy labelling was visible throughout their walls The extravascular blackening was also noted in most experimented animals Bloody fluids were obtained in all cases Cytology revealed the

min It was a typical transudate—a thin, ear yellow fluid

It is likely that negative pressure applied to ie middle ear creates an abnormally low ydrostatic pressure causing small blood ves els (venules and many of capillaries) to pro uce an exaggerated amount of ultrafiltrate or ransudate Additionally, mast cells, which re distributed in the normal middle ear mu osa, may participate in increasing vascular ermeability (Hiraide & Paparella, 1972) The nast cells are stimulated by changing atmopheric pressure and then histamine or another nediator is released, causing vasodilation and eakage of regional vessels

As demonstrated in an animal experiment y Lamkin et al (1975), positive as well as ne gative pressure influences the middle ear nucosa with occasional transudation of serous luid and hemorrhage This suggests that posi ive pressure can stimulate mast cells in the niddle ear mucosa to release chemical me liators such as histamine, serotonin, and others, which subsequently cause the vaso dilation and production of middle ear fluid

Persistent negative pressure leads first to tympanic edema and then transudation Trans udation of blood serum from capillaries and venules into the tympanic cavity may take place in tubal occlusion due to absorption of oxygen as soon as the pressure state in the middle ear cavity falls below that of the plasma protein osmotic pressure Several investigators have measured the under pressure of the diseased human middle ear cavity (Dis hoeck, 1941, Perlman 1943, Thomsen, 1958) They found negative pressures varying down to a maximum of −60 cm H_2O and presence of middle ear fluids

Holmgren (1940) also measured negative pressure equivalent to 5–7 cm H_2O in the tym panic cavity in dogs, of which the Eustachian tube was operatively occluded

Matsumura (1955) analysed the middle ear gases of cats after cauterization of the pharyn geal orifice of Eustachian tubes He found direct correlation between the degree of tubal obstruction and depression of oxygen levels

Elner (1976) has calculated the rate of gas absorption from the human middle ear He has determined the mean value to be 33 μl/l hour or 0 7–1 1 ml/24 hours under normal conditions On the basis of recordings of the pres sure drop in the ear in cases of perforated tym panic membrane, Riu et al (1966) have calculated the absorbed volumes and found approximately the same value (0 8 ml/24 hours)

The ear is a nearly rigid chamber which can decrease its volume very little only by inward movement of the tympanic membrane When the Eustachian tube is closed the middle ear cavity becomes a closed chamber Gas ab sorption occurs gradually and the underpressure grows in the closed middle ear cavity When this reaches a certain limit, transuda tion occurs due to the increase in vascular permeability

After experimentally obstructing the Eus tachian tubes, an obvious increase in vascular permeability invariably occurred in the sub mucosa of squirrel monkeys and guinea pigs (Paparella et al , 1970, Hiraide & Paparella, 1972)

Accordingly, transudate from capillaries is the main component of the fluid in the early stage of serous otitis media

We are of the opinion that the blood vessels undoubtedly participate in the production of the middle ear fluids in earo-otitis media and presumably in serous otitis media

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

Durch lokale Applikation des negativen Druckes von vielerlei Graden am Mittelohr eines Meerschweinchens beobachteten wir die Veranderungen der vaskularen Per meabilitat nach der Majnoschen vaskularen etikettieren den Technik Im experimentellen Mittelohr waren zu nehmende Permeabilitat und Effusionen gefunden Alle

Drucke unter −5 mmHg veranlassen die Mittelohreffusion Also die Absteigerung des Druckes in der Mittelohrhohle mag die Transudation des Serums von subepithelialen Gefäßen verursachen welche aeroottische Flussigkeit ergeben hat Im solchen Zustande mogen die Mastzellen vom Druck der Atmosphäre leicht beeinflußt in der zunehmenden vaskularen Permeabilitat enthalten werden

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DIE LACTATDEHYDROGENASE (LDH) DES INNENOHRES NACH LARMBELASTUNG

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(Eingegangen am 8 November, 1976)

Abstrakt Diese Abhandlung beschreibt die histologischen und biochemischen Studien der LDH Vorgange des Cortiorganes der Stria Vascularis und der Perilymphe unter akustischer Uberreizung Die Tiere wurden uber einen Zeitraum von 10 min bis 72 Std mit einem „Rosa Rauschen einer Frequenz von 50 Hz bis 10 KHz in einem diffusen Schallfeld beschallt. Der Larm pegal betrug in jedem Fall 115 dB Nach Dauerlarmeinwirkung von einer Stunde konnte eine eindeutige Abschwachung der Farbung in den innern u ausseren Haarzellen sowie an den dazugehorigen Nervendigungen festgestellt werden Wenn die Schallbelastung jedoch aus 15 Stunden ausgedehnt wurde zeigte sich Erhohung der LDH Aktivitat Eine biochemische Untersuchung folgte der oben beschriebenen histochemischen Arbeit Die LDH Aktivitat des Cortiorganes und der Stria Vascularis der einzelnen vier Windungen wurde mit der sogenannten mikrobiochemischen Technik gemessen

Diese Abhandlung beschreibt die histologischen und biochemischen Studien der LDH Vorgange des Cortiorganes, der Stria Vascularis und der Perilymphe unter akustischer Uberreizung

Seit der bekannten Arbeit von Wittmaack (1907) gibt es viele histopathologische Untersuchungen des Innenohres unter Larmbelastung Neue Aspekte in der Beurteilung der Stria Vascularis und des Cortiorganes bei Larmschadigung bringt die Betrachtung biochemischer Vorgange Es liegen nur wenige Ergebnisse histochemischer Untersuchungen der LDH vor Fermentlokalisationen in der Cochlea bei Larmschadigung wurden von Vosteen (1961 und 1964), Koide (1964), Gerhardt (1964) und Vinnikov & Titova (1963) vorgenommen

Vosteen (1961) bemerkte als Erster einen graduellen Abfall der SDH nach Lärmbelastung, beginnend zuerst in den Nervenendigungen, dann in den ganzen äusseren Haarzellen Ähnliche Befunde bekamen Vinnikov & Titova (1963) beim Meerschweinchen und Kaninchen Vosteen interpretierte diese Veranderungen als Ausschopfung des aeroben Metabolismus Er vertrat die Ansicht, dass neben dem aeroben glykolytischen Metabolismus ein anaeroben Weg existieren musste, der als Notfallreserve bei kurzer Ubereinstimmung oder kurzzeitigem Sauerstoffmangel zur Verfügung stehe Eine Reihe wichtiger Arbeiten auf die quantitative biochemische Bestimmung im Bereich des Innenohres gerichtet Rauch (1964), Rauch & Plester (1965) Schindler et al (1965), Schindler & Schnieder (1966), Silverstein & Schuknecht (1966), Lotz & Kuhl (1968), Matschinsky & Thalmann (1967), Thalmann & Matsehmsky (1970) Quantitative Angaben uber die Pyridinnucleotide in der Stria Vascularis bringt Matschinsky & Thalmann (1967) Von Iimura (1967) liegen Ergebnisse über die ATPase in der Stria Vascularis und dem Ligamentum spirale vor

Um einen Begriff von der Grosse des energieliefernden Stoffwechsels zu bekommen, wurde die LDH in den Stria Vasculari der

einzelnen vier Windungen und dem Cortiorgan der Meerschweinchencochlea untersucht

METHODIK

Es wurden 47 bunte gut horende Meer schweinchen mit einem Gewicht von 240–360 g verwandt Die Tiere wurden uber einen Zeitraum von 10 Min bis 72 Std mit einem ,Rosa Rauschen einer Frequenz von 50 kHz bis 10 kHz in einem diffusen Schallfeld be schallt Der Larm pegal betrug in jedem Fall 115 dB Bei allen Tieren wurden direkt nach Beschallungsende in einer Nembutalnarkose durch retroaurikulares Vorgehen die Cochlea freigelegt und je zwei μl Perilymphe durch das Runde Fenster entnommen Unmittelbar nach der Perilymphentnahme erfolgte die Dekapita tion der Tiere und Entnahme beider Coch leae Die Cochleae wurden unter kalter phy siologischer NaCl Losung eroffnet und die Stria Vascularis sowie die Cortiorgane ein schliesslich der unmittelbar angrenzenden knochenfreien Strukturen der einzelnen vier Windungen separat zur anschliessenden spek tro photometrischen Bestimmung entnom men Die Proben wurden nach ihrer Entnahme glasernen Mikrorohrchen in kalter physio ischer NaCl Losung eroffnet homogenisi rt und nach Zentrifugieren der klare Uber stand für die folgende Bestimmung des Ge samteiweisses und der LDH Aktivitat abpipet tiert Das Gesamteiweisse wurde nach der Folin Methode bestimmt Zur spektro-photo metrischen Messung diente ein Zeiss spektral photometer PMQ II

Die Kontrolluntersuchung wurden an 14 weiteren Tieren ohne Schallbelastung ermit telt Anschliessend entfielen zwei Tiere zur morphologischen Untersuchung auf jeden Beschallungsversuch Die Schnecken wurden wie oben beschrieben von retroaurikular auf gesucht und supravital nach Fixieren des Sta pes mit kaltem Karnovsky schen Fixations gemisch (1965) von Runden Fenster aus per fundiert Nach ihrer Entnahme nd Nachfixa tion in dem gleichen Gem h erfolgte die hi stologische Aufarbeitung in ... technik nach Barker Das Eindrangen des Inkubationsge misches durch regelmassige Schaukelbewe gungen bei 37°C erleichtert Schliesslich wur den die mit 10% EDTA entkalkten Prapara.. in Paraplast eingebettet und die angefertigten Schnitte unter einem Zeiss Forschungsmikro skop beurteilt

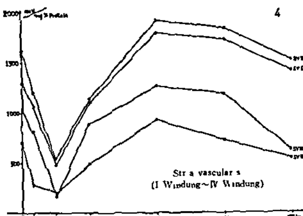
ERGEBNISSE

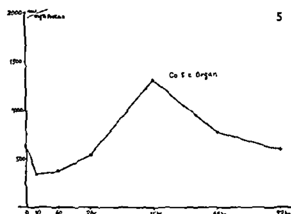
Als histochemisches Ergebnisse bei den nor malen Tiere ohne Schallbelastung kam LDH im grossen Umfang in den Haarzellen wobei kein Unterschied in der Aktivitat aussern und innerer Haarzellen erkennbar war Allerdings lagen die Formosanablagerung in den inneren Haarzellen relativ gleichmassig verteilt im ge samten Cytoplasma Dagegen wurden etwas kraftigere Ablagerungen vom Farbstoff an der Zone des Nervenendigunges am unterer Zell pol der aussern Haarzellen (Abb 1) Nach Dauerlarmeinwirking von einer Stunde konnte eine eindeutige Abschwachung der Farbung n den innern u ausseren Haarzellen sowie an den dazugehoriges Nervenendigungen festge stellt werden (Abb 2) Wenn die Schallbe lastung jedoch auf 15 Stunden ausgedehnt wurde zeigte sich Erhohung der LDH Aktivi tat (Abb 3)

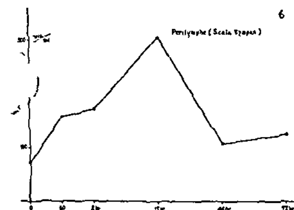
Eine biochemische Untersuchung folgte der oben beschriebenen histochemischen Arbeit Die LDH Aktivitat des Cortiorganes und der Stria Vascularis der einzelnen vier Windung en wurde mit der sogenannten mikrobioche mischen Technik gemessen Bei normalen Meerschweinchen zeigten biochemisch die Cortiorgan und Stria Vascularis keinen Aktivi tatsunterschied der LDH erkennbaren

Aus den Kurvenverlaufan wobei auf d Ordinate mU/mg Eiweiss und der Abszisse de Zeitverlauf aufgetragen sind ist zu entnehm en dass in den ersten Stunden die LDH Ak tivitat der Stria Vascularis der einzellnen vier Windungen abnimmt nach diesem Zeitraum jedoch sich eine steigende Tendenz zeigt Nach 15 Stunden kommt es wieder zu einem massigen Aktivitatsabfall Der Kurvenverl für die einzellnen Windungen erscheint paral


1
2
3







lel, wobei die Werte aus der 2te Windung den hochsten Kurvenverlauf ergeben gefolgt von der ersten, dritten und vierten (Abb 4)

Die Kurve des Cortiorganes verlauft ahnlich wie bei der Stria Vascularis Am Cortiorgan finden wir auch innerhalb der ersten 10 Minuten einen starken Abfall der LDH Aktivitat, gefolgt von einem Anstieg der Aktivitat, der der nach Beschallung von 15 Stunden sein Maximum erricht Danach zeigt an die LDH einer massigen Abfall der Aktivitat (Abb 5

Weiter wurde die LDH-Aktivitat in der Pe lymphe nach Beschallung untersucht Na Schallbelastung von 15 Stunden fand sich e Maximumwert der LDH, 5 mal so hoch w Kontrollwert Bis zu 15 Stunden nach d Beschallung zeigte sich eine steil aufsteigen Linie ohne Abfall in der ersten Stunde D nach weist die starken Abfall wieder auf Na mehr als 48 Stunden Beschallung zeigt die A tivitatskurve einen annaherend ebenen Ve lauf, der bei Kontrollwerte entsprechend (Abb 6)

DISKUSSION

Der Glucoseabbau im Innenohr erfolgt oxy tiv uber den Krebszyklus (Citronensaurez lus), nachdem zunachst glycolytisch Bre traubenzaure gebildet worden ist Brenztr benzaure kann zu Milchsaure hydriert werd so dass als Endprodukt des glycolytisch Glucoseabbaues im Innenohr das Lactat gesehen werden kann Dieser Lactatanteil tragt, bezugen auf die Gesamtmenge der a genommenen Glucose 7–8% Nach den U tersuchungen von Barkuli werden zwei Dri der im Gehirn zur Oxydation zur Verfüg stehenden Glucose zunachst in Aminosä uberfuhrt, wahrend das restliche Drittel dir und vollständig zu CO_2 veratmet wird T Gewinn bei Glycolysis betragt zwer nur 2 A pro mol Glucose, aber viel mehr Energie mag die Umwandlung in Milchsäure nicht liefern Ausserdem besitzen wir noch ein haltnismassig energiereiches Substrat – Milchsaure – zum weiteren Abbau, w Sauerstoff verfugbar ist Lactat bewirkt im wie ein Vasodilatator und damit ein Aufre erhalten der Durchblutungsgrosse Fehlt doch der Sauerstoff im Wirbeltierorganism bei der anaeroben Glykolysi des Musk wird Pyruvat zu Lactat reduziert

In Analogie zu den Befunden Thema (1963) an der Netzhaut schloss Vosteen eine Abhängigkeit von der unterschiedlic

rt der Energiegewinnung durch uberwiegend naerobe (LDH) und aerobe Fermentausstattung (SDH) Als Fazit dieser Untersuchungen esultiert eine Gleichung (Schatzle, 1971) ute Durchblutung=gute Sauerstoffversorgung=uberwiegend aerober Stoffwechsel (Basale Schneckenwindung, innere Haarzellen)= geringereer Glycogengehalt und geringe oder ehlende Ausstattung mit anaerobtatigen Fermenten

Schlechtere Durchblutung=schlechtere Sauerstoffversorgung=potentiell anaerober [illegible] eckenwindungen, [illegible] Glykogengehalt und gute Ausstattung mit anaerob tatigen Fermenten

Einen weiteren Beweis, dass aerobe und anaerobe Prozesse bei der Larmbelastung eine Rolle spielen, lieferten Hayata & Harada (1967) Sie untersuchten Aktivitat des SDH und Phosphorylase, das erste Enzym als Parameter des aeroben und das letztere als Parameter des anaeroben Metabolismus Nach Beschallung mit 100 dB für 3 Stunden konnten Conti & Borgo (1964) eine diffuse Abnahme der Zytochromoxydase im Cortiorgan unabhangig von den benutzten Frequenzen nachweisen Nach mehrtagiger Beschallung mit einem Dauerton liess sich eine Abnahme der SDH-Aktivitat in der Meerschweinchenkochlea beobachten (Vosteen, 1958, 1960*a*, 1961) Eine zunehmende Beschallungsdauer mit 2 000 Hz und 70–85 dB bewirkte eine zunehmende Ausbreitung, des betraffenen Bezirks der zweiten Windung Im Zentrum des Ansprechgebietes kamen auch histologische Schadigungen vor Die Abnahme der Fermentaktivitat erfolgte zunachst in den Nervenendigungen dann in der ganzen ausseren Haarzelle Die inneren Haarzellen bleiben unberuhrt Eine Erholung nach Dauerbeschallung trat innerhalb der Nachbeobachtungszeit von 5–6 Wochen nicht ein Bei Beschallung mit Kurztonen uber 2–4 Tage fanden sich nur ausserordentlich geringe Veranderungen Die Schallschadigung des Cortiorgans soll somit nicht Folge einer mechanischen Zerstörung von Haarzellen, sondern die letzte Stufe einer Stoffwechselerschopfung der Phonorezeptoren sein Nach beschallung beschreiben auch Vinnikow & Titova (1963) eine Abnahme von SDH in den ausseren Haarzellen und Spiralganglionzellen von Meerschweinchen und Kaninchen

Viele Autoren untersuchten die Sauerstoffabnahme in den endolymphatischen Flussigkeiten nach Schallbelastung (Misrahy & Hildreth 1958, Koide 1960) Misrahy machte zwei verschiedene Effekte fur diesen Sauerstoffmangel verantwortlich Ein Anwachsen des Sauerstoffverbrauches durch metabolische Haarzellprozesse und die Verminderung der O_2 Diffusion von der Stria Vascularis in die Endolymphe unter Larmbelastung sprach weiterhin für diese Idee

Yamashita (1973) hat schon die LDH und SDH Aktivitat am einzelnen Cortiorgan unter verschiedenen Sauerstoffspannungen gemessen und festgestellt, dass über 60 M der arobische Atmangstoffwechsel nicht wirkt, wo dagegen das anarobische Glycolyse starke Wirkung zeigt

Eine Anderung der Aktivität eines Enzyms in einer Korperflussigkeit oder in einem Organ verschiedene Ursachen haben

1 Die Grundsubstanz, an die das Enzym gebunden ist, geht zu Grunde und das Enzym wird mit dem Blut oder der Lymphe aus geschwemmt
2 Die Permeabilitat der Zellmembran ändert sich Um die Membranpotentiale und Konzentrationsunterschiede aufrecht zu erhalten, verbraucht die Zelle laufend freie Energie
3 Durch eine Änderung des Zellstoffwechsels kommt es zu einer vermehrten Produktion von Enzymen

Die Änderung der LDH Aktivität nach Beschallung konnte auch durch die obengenannten verschiedenen Prozesse verursacht werden Der LDH Abfall nach einstundiger Beschallungsdauer, der eine Zunahme der aeroben Glykolyse ausgewiesen haben konnte, ist

deutlich signifikant in der 1 und 2 Windung gegenuber den Ausgangswerten (Kontrollwerten), wahrend diese Signifikant fur die 3 und 4 Windung nicht so deutlich ist Werden die Tiere langer als 60 Minuten beschallt, so kommt es zur Zunahme der LDH-Aktivitat, die einem aussteigenden anaeroben Stoffwechsel andeuten konnte

ZUSAMMENFASSUNG

Die Schallwirkung auf die LDH Aktivitat des Innenohres war Gegenstand dieser Arbeit An Meerschweinchen wurde der schadigende Einfluss von Larm auf die LDH-Aktivitat der Stria Vascularis, des Cortiorgans und der Perilymphe mit Hilfe biochemischer Methoden untersucht, die eine quantitative Auswertung der Befunde erlauben

In der Stria Vascularis nimmt die Gewebeaktivitat der LDH in den ersten Stunden ab, nach dieser Zeit zeigt sie eine steigende Tendenz Nach 15 Stunden kommt es jedoch zu einem massigen Aktivitatsabfall Der Kurven ...auf fur die einzelnen Windungen erscheint ...llel, wobei die Ergebnisse aus der 2 Win...g den hochsten Kurvenverlauf zeigen, dann kommt in dieser Reihenfolge, die erste, dritte und vierte

Im Cortiorgan finden wir innerhalb der ersten 10 Minuten nach Beschallung einen sehr starken Abfall der LDH Aktivitat, die nach 15 Stunden Beschallung ihr Maximum erreicht

Im Bereich von 15 bis 48 Stunden Beschallung kommt es parallel zu den Befunden in der Perilymphe zu einem sehr starken Abfall der Aktivitat Nach mehr als 48 Stunden Beschallung finden wir nur noch eine sehr massige Aktivitätsabnahme im Cortiorgan Bei der Untersuchung der Perilymphe bis zu 15 Stunden Beschallungsdauer sieht man eine steil auf steigende Linie, die danach wieder einen starken Abfall aufweist Nach mehr als 48 Stunden Beschallung verlauft die Aktivitats kurve fast flach, die dem Kontrollewert entsprach

SUMMARY

This paper deals with histochemical and biochemic studies on LDH activity in the organ of Corti the str vascularis and the perilymphatic fluid in the inner e under acoustic overstimulation

Forty seven normal guinea pigs with good Preyer reflex were used These animals were continuously ex posed to white noise of 115 dB for periods ranging fr 10 minutes to 72 hours After acoustic overstimulatio the inner ears were exposed and perfused with LDH in cubation solution through the scala tympani to the sca vestibuli The animals were decapitated and the inne ears were fixed with Karnovsky s solution and embedde with Paraplast LDH staining of the Corti organ and str vascularis showed formation of formosan When expose for 60 minutes almost all the formosan was found t have disappeared However when exposed for 15 hour the LDH activity was found to have increased again

A biochemical study followed the above described his tochemical study The LDH activity in the Corti orga and every turn of the stria vascularis was measured b the so-called microbiochemical technique The animal were decapitated and the inner ears were placed in phy siological saline at about 4°C The Corti organ and str vascularis of each turn were obtained separately by th surface preparation and homogenized in fine glass tube The homogenate obtained was centrifuged and the super natant fluid was assayed for LDH activity and prote content In normal guinea pigs the organ of Corti an *all parts of the stria vascularis proved to have alm* the same level of activity

Of the four turns of the stria vascularis the secon turn was shown to have the highest activity and to b followed by the first third and fourth in decreasing order

When exposed for 60 minutes the activity decrease markedly in all the turns of the stria vascularis and con stituted the lowest levels When exposed for 15 hours the activity attained the highest levels After 72 hours of ex posure however the activity decreased again and re turned to the normal levels

The curve for the Corti organ followed the same pa tern as in the stria vascularis In the case of perilymph the activity peaked at 15 hours (five times the norma level) and then decreased gradually to normal

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THE SIZE OF THE MIDDLE EAR AND THE MASTOID AIR CELL
System measured by an acoustic method

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Abstract The total volume for the middle ear and mastoid air cells was measured in 55 temporal bones with normal ear drums from a Norwegian post mortem material The measurements were made by an acoustic method and checked by means of fluid filling induced by vacuum pumping As a measuring fluid X ray contrast medium was employed so that the degree of filling could be examined by X ray The volume varied between approximately 2–22 cm³ with an average round 6 5 cm³

It is a common observation that both the extent and the composition of the mastoid air system can have great individual variations, and that it differs on the right and left sides in the same individual (Silbiger, 1950) This is in accordance with our observations

A system with many small air cells has a greater surface area than one with fewer, larger cells, when the volume is identical It follows that a mastoid air cell system with larger cells should function better as a reservoir than a system with small cells

Holmquist & Miller (1973) have studied the Eustachian tube and the middle ear function in the Rhesus monkey They found that sudden pressure changes such as found in flying and diving, will stress the Eustachian tube function more in an individual with a big mastoid air cell system than in one with a smaller system

Flisberg et al (1963) used Boyle s law for their clinical volume determinations of the air filled ear space

Ingelstedt et al (1967) have earlier described a method for determination of volume of the air filled middle ear space persons with intact ear drums

This investigation has had two chief aim

1 To measure the middle ear and mastoid air cell volumes in a Norwegian autopsy material

2 To develop a method of measurement which can be employed clinically to patients with perforated ear drums without causing undue discomfort to the patient

MEASUREMENTS

The volume of an enclosed quantity of can be determined acoustically by measu the change in pressure, i e sound press when the volume is given a small harm (sinusoidal) change The change in vol must occur so gradually that the change pressure has time to adjust itself through the volume evenly In other words, the so frequency must be so low that the cross mensions of the volume are small in rela to the wavelength

It can then be shown that the sound p sure will be proportional to the relative cha in volume and independent of the frequenc

$$p = \text{const} \cdot \frac{1}{V}$$

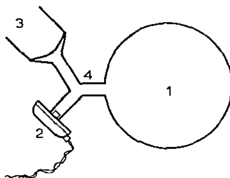


Fig 1 Arrangement for acoustical volume measurement. 1 Volume to be measured 2 Hearing aid telephone 3 Microphone 4 Y tube

where p = sound pressure
v = change of volume
V = the volume to be determined

For the *same* change of volume in measuring two different volumes V_1 and V_2 the relation between corresponding sound pressures p_1, and p_2 will be

$$\frac{p_1}{p_2}=\frac{V_2}{V_1} \quad (2)$$

If V_1 is a known reference volume V_2 can be determined by

$$V_2=V_1\frac{p_1}{p_2} \quad (3)$$

Changes in volume can be obtained by using an ordinary magnetic hearing aid telephone A constant excitation current to the telephone will cause the movement of the membrane to give a constant change of volume over a sufficiently large range of the measuring volume and frequency The measuring arrangement is shown schematically in Fig 1

The supplementary volume V_e caused by the microphone, telephone and connecting tube can be determined by eq (2) by measuring the pressures p_1 and p_2 in two known volumes V_1 and V_2

$$\frac{V_2+V_e}{V_1+V_e}=\frac{p_1}{p_2}$$

i e

$$V_e=\frac{V_1\frac{p_1}{p_2}-V_2}{1-\frac{p_1}{p_2}}$$

Eq (3) can now be modified to

$$V_x=(V_r+V_e)\frac{p_r}{p_x}-V_e \quad (4)$$

where V_x = the volume to be determined
V_r = the known reference volume
p_x = the sound pressure in V_x
p_r = the sound pressure in V_r

The frequency curve for the sound pressure is recorded to control that the response is flat at low frequencies In the volume determinations the mean of the measured levels at 30 and 50 Hz was employed

MATERIAL

See tabular survey in the appendix (Table II) Human temporal bones with normal ear drums were cleansed of soft tissue A Y tube was placed in direct contact with the middle ear or mastoid air cells and cemented fast with Grip dental cement In some of the preparations, in addition to the placing of a direct tube, a tube was also inserted through a plug in the ear canal In these preparations my-

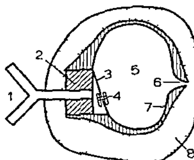


Fig 2 Y tube placed in the ear canal *1* Y tube *2* Neoprene plug *3* Ear drum *4* Ventilation tube *5* Middle ear *6* Eustachian tube *7* Medial bony wall of middle ear *8* Wax

ringotomia was performed, and in some cases a plastic ventilation tube was inserted

The chance to get reliable measurements through the tube placed in direct contact with the cell system we wanted to measure, should be optimal

But we also wanted to know whether we could get reliable measurements through a tube placed in the ear canal when there was a perforation in the ear drum If so the method would satisfy the second chief aim of this investigation (q v) Since this tube ended in the ear canal it was not in direct contact with the volume we wanted to measure Consequently we called this tube placing "indirect"

The following methods of tube insertion were employed

I Direct placing

(*a*) Through the tegmen tympani
(*b*) Through the bulbus superior venae jugularis internae
(*c*) Through the apex precessus mastoidei

II Indirect placing

(*a*) In the ear canal in combination with myringotomia
) In the ear canal in combination with a ventilation tube in the ear drum (Fig 2)

The tube in the ear canal passed thorugh a neoprene cellular rubber plug which filled the ear canal right up to the ear drum (The small volume which might have remained between the plug and the ear drum in connection with these measurements constitutes so small a proportion of the total volume that it has been left out of account)

The preparations were then dipped in melted paraffin wax several times until they were sealed within a thick airtight layer (Fig 3)

33 of the preparations were X rayed before measurement to obtain information concern ing (*a*) the degree of pneumatization which affords an approximate idea of the volume (*b*) the size of the air cells (*c*) to examine the placing of the tubes (Fig 4) and (*d*) to ensure



Fig 3 Preparation No 38 enclosed in wax Y tube pl in the ear canal

that the cell system was intact without path logical alteration or artefact

All preparations were first measur acoustically To remove possible fluid ma of the preparations, after the first acoust measurement, were vacuum pumped befo being subjected to a new acoustic measur ment as a check (See appendix)

In order to check our acoustic measur ments, all preparations were also measured b means of fluid filling The air in the prepar tion was first pumped out and replaced wi fluid from a graduated tube, so that the volu of fluid which was sucked in could be re off The arrangement for measurement shown schematically in Fig 5

The air in the preparation, and the ext air because of the Y-type and additional tu is removed by means of a vacuum pump wh is then closed at stopcock 3 Stopcock opened and the volume of fluid drawn in (is read off from the graduated tube The



Fig 4 Pre filling X ray control One tube through tegmen tympani the other placed in the external ear canal Internal porus acousticus and the cochlea are seen near the apex of the pyramid

nown volume V_x is determined by subtract ıg the extra volume V_e

$$V_x - V_r - V_e \quad (5)$$

An air pressure of 1 5 torr was produced by pumping The remaining air will therefore result in only about 2 ‰ too little fluid being drawn in This is a negligible error As measuring fluid was employed the X ray contrast medium Isopaque Cerebral Nyco 280 mg I/ml diluted with 1 part contrast 2 parts water so that the degree of filling could be supervised by X ray Finally, the preparations were opened and checked under an operation microscope to ascertain whether there was any damage of the ear drum the annular ligament and the membrana fenestra cochleae with possible leakage of the contrast fluid outside the air filled cavities

RESULTS

All the primary measurements are given in the Appendix The results are summarised in Table I to provide a synopsis and a basis for comparison of results The volume distribution of 39 temporal bones measured acoustically after vacuum pumping is shown in Fig 6

DISCUSSION

Littler (1965) reports following Zalewski that the breaking strength of ear drums of normal appearance in post mortem preparations is 0 4–3 0 atmospheres The mean value for 111 ears was 1 6 atmospheres In our experiments the middle ear pressure was reduced to approximately 1 5 torr (1 atmosphere=760 torr) The ear drum will then move inward somewhat towards the middle ear, which will

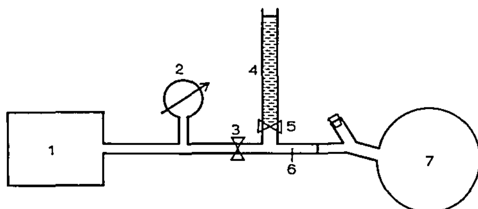


Fig 5 The arrangement for fluid filling *1* Vacuum pump *2* Pressure gauge *3* Stopcock *4* Graduated, fluid filled tube *5* Stopcock *6* Connecting tube *7* Unknown volume (middle ear and mastoid air cells)

result in an inconsiderable reduction in volume there (Fig 7) The same reaction of the ear drum will meanwhile cause a relatively great change in volume in the small amount of air trapped outside the ear drum This involves a relatively large decrease in pressure in this space also, simultaneously with the fall of pressure in the middle ear, and the difference in pressure across the ear drum will thus be comparatively small

It might be supposed that the considerable fall in pressure in the middle ear might cause rupture of the anular ligament and/or the membrane of the fenestra cochleae However, since the theoretical tensile strength of pure water exceeds 1 000 atmospheres, the fluid in the inner ear cannot expand Simultaneous reduction of the pressure upon the fenestra cochleae and the fenestra vestibuli will thus be unable to dislocate these outwards, so that they burst

On opening the preparations and examining them under the operation microscope, it was also apparent that these structures had withstood the pumping The ear drum had burst however, in two of the preparations

In the acoustic method the placing of the measuring tube through the ear canal has afforded very nearly the same results as when

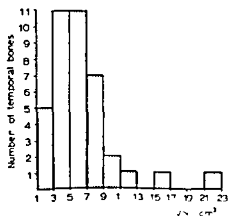


Fig 6 The volume distributi n (3) temporal bones measured acoustically after v u n pumping

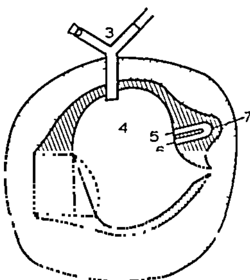


Fig 7 Vacuum pumping of middle ear *1* Small amount of air trapped outside the ear drum *2* Ear drum bulged somewhat inward during vacuum pumping *3* Y-tube connected to vacuum pump *4* Middle ear *5* Fenestra vestibuli *6* Fenestra cochleae *7* Cochlea

Table I *Some different ways of presenting the results*

Data definition	No of data	Volume of middle ear and airfilled spaces of mastoid process [cm³]			
		Mean	Median	Quartiles	
				Lower	Upper
AM BP DIR	19	6 3	4 8	4 1	6 2
AM AP DIR	19	7 0	5 2	4 6	7 1
LM (1)	19	6 6	5 4	4 4	7 0
LM (10)	19	7 2	6 2	4 6	8 1
LM (∞)	19	7 5	6 8	4 9	8 3
AM BP DIR	22	6 0	4 8	4 1	6 6
AM BP EC	22	6 1	4 8	4 1	6 7
AM AP DIR	23	6 4	5 1	4 3	6 9
AM AP EC	23	6 5	5 1	4 4	6 9
AM PB	51	6 1	4 9	3 7	7 6
♂	22	5 9	5 2	3 6	6 8
♀	27	6 0	4 9	3 9	7 9
LM (∞)	46	6 9	6 0	4 5	8 3
♂	21	6 4	5 3	4 0	7 3
♀	23	7 2	6 8	4 9	9 7
AM BP DIR	34	5 9	4 9	3 9	6 6
AM AP DIR	34	6 5	5 2	4 4	7 6
LM (∞)	34	6 8	6 0	4 3	8 1
AM BP DIR	39	5 6	4 8	3 6	6 7
AM AP DIR	39	6 2	5 1	3 9	7 6

Abbreviations AM Acoustical Method LM Liquid Method BP Before Pumping AP After Pumping EC measuring tube through Ear Canal DIR measuring tube inserted DIRectly into volume (1) (10) (∞) indicate results with LM after approximately 1 10 seconds and a time long enough to give stationary values

the measuring tube is inserted directly into the middle ear or mastoid air cells (see point 2 in Table I) Volume seems to have little connection with sex (see points 3 and 4 in Table I)

In acoustic determination of volume, it was a necessary presupposition that measured sound pressure in the volume should be independent of the frequency within a limited frequency range If this also proves to be the case in practice, it will provide a strong indication that the correct volume has been measured, viz , the volume of the middle ear itself and of the air filled mastoid cells If, on the other hand the pressure increases with the frequency, that could indicate that more and more small part volumes are shut off from the rest of the total volume This could be due to the mass inertness of the air, or through possible fluid membranes, which in the narrow connective canals hinder the levelling out of pressure as the frequency increases

Only a very few measurements have been omitted from our material on account of variation in the sound pressure within the actual frequency area (30–50 Hz)

The results derived from measurement by fluid filling are more difficult to interpret The sucking in process happens rapidly at first, then more slowly The indiviual variations in this process are wide On the average, approximately 90% of the fluid will be drawn in during the first second, after 10 seconds, 96% will be drawn in, and the remainder in the course of 3–5 minutes The total volume will, on average, be greater than when measured acoustically This can be explained as being due to the opening of obstructed part volumes by the pumping out process The obstruction can for example be caused by fluid membranes

Table II *Results (volume measured in cm³)*

Prep no	Sex	Age	Side	Acoustic method				Liquid method Filling time (sec)		
				Before pump		After pump				
				Dir	E c	Dir	E c	1	10	∞
1	?	?	?	4 9						4 7
2	?	?	?	13						12 2
3 }	♀	71	R	7 8						–
4 }		71	L	4 9						–
5 }	♀	72	R	11 0						11 7
6 }		72	L	11 9						–
7 }	♀	75	L	11 0						11 8
8 }		75	R	–						10 4
9 }	♂	82	R	7 8						6 7
10 }		82	L	7 3						7 6
11	♂	74	R	–						–
12 }	♂	68	R	3 0						4 3
13 }		68	L	3 6						4 7
14 }	♂	88	R	6 7	6 8	7 8	7 8			–
15 }		88	L	4 0						5 3
16 }	♂	57	R	9 6		10 5				10 0
17 }		57	L	9 3		10 2				9 6
18 }	♀	68	R	8 7		8 6				9 8
19 }		68	L	5 2		7 0				6 3
20 }	♀	50	R	4 6		5 0				5 7
21 }		50	L	7 9		8 5				8 3
22 }	♀	49	R	2 2	2 2	2 3	2 2			2 2
23 }		49	L	2 5		3 6				3 9
24	♂	76	R	6 7		7 8				7 9
25 }	♂	69	R	–		–				–
26 }		69	L	3 5		3 2				3 8
27	♂	69	R	5 7		5 7				5 6
78 }	♂	83	R	3 6		4 3				3 8
}		83	L	4 9	5 1	5 1	5 3			5 3
}	♂	82	R	2 5		2 2		1 7	1 9	3 2
,}		82	L	2 9		3 0				2 9
	♀	84	R	6 4	6 5	6 9	7 1	6 0	8 4	8 7
3	♂	65	L	1 8	1 8	2 2	2 2	2 0	2 2	2 5
34	♀	65	L	5 7		8 1		8 4	9 5	9 6
35 }	♀	95	R	3 3	3 3	3 6	3 1	2 6	3 1	–
36 }		95	L	4 0	4 0	4 4	4 5	3 6	4 6	4 9
37	♂	54	R	4 7	4 8	5 1	5 2	4 5	4 55	4 6
38 }	♀	82	R	9 7	9 7	11 0	11 3	9 6	10 5	10 8
39 }		82	L	14 1	14 2	15 1	15 4	11 7	12 2	12 6
40 }	♀	69	R	1 6	–	2 1	–	2 0	2 3	2 6
41 }		69	L	1 3		1 9		–	–	–
42	♀	84	R	4 1	4 2	4 9	5 0	4 7	4 8	5 3
43	♂	75	L	6 8	6 8	6 9	6 6	–	–	–
44	♂	73	L	5 5	–	5 9	–	6 1	6 4	7 0
45	♀	84	L	5 9	6 1	5 8	6 4	5 8	6 1	6 9
46 }	♂	74	R	–	–	3 4	3 5	3 3	4 0	4 2
47 }		74	L	4 [illegible]	4 1	5 3	5 5	5 2	5 5	5 7
48	♂	49	L	5 9	6 0	6 6	6 6	6 7	7 5	7 6
49	♀	64	L	7 2	7 4	7 3	7 5	7 2	7 7	7 9
50	♀	71	L	3 7	3 9	4 8	4 7	5 6	6 6	6 9
51	♂	75	L	19 2	19 0	21 0	22 0	21 9	22 3	22 4
52 }	♀	74	R	4 3	4 3	4 2	4 3	2 7	2 8	2 9
53 }		74	I	4 1	4 1	4 6	4 6	4 5	4 9	4 9
5' }	♀	70	R	4 4	4 4	4 5	4 5	4 3	4 6	5 2
55 }		70	I	4 8	4 8	5 0	5 0	4 5	6 2	6 7

Abbrev Dir Direct E C Far canal Brackets show paired preparations



Fig 8 Specimen No 41 anterior–posterior projection Tomographic cut after contrast filling of this temporal bone visualize the internal acoustical porus, the vestibulum the lateral and superior semicircular canal, the oval window, stapes, incus and Y tube through tegmen tympani Contrast fluid filling is very poor Possibly a thin shift of contrast fluid is located between the corpus of incus and the lateral semicircular canal

fluid plugs in the connective canals between : part volumes Seen in this light, the agree- :nt between the methods must be regarded satisfactory, and the fluid method with exa nation by X-ray of the degree of filling has nfirmed the usefulness of the acoustic ethod

Our measurements show a mean volume of proximately 6 5 cm³ The values varied ap- oximately between 2 and 22 cm³ (cf Table)

Silbiger (1950) found in his material from asel mean values around 9 5 cm³ (varying om 2 6 to 20 9 cm³)

Zwislocki (1962) found in a limited material iean values round 8 6 cm³ (2 1 to 17 4 cm³)

Flisberg et al (1963) found variations from 0) 25 cm³

Flisberg & Sigmond (1965) found in their iaterial from Lund mean values round 12 2 m³ in healthy ears

Andreasson et al (1976) have shown how he vascular bed (blood and lymph) in the nucous membrane influences on the volume f the middle ear when ambient and/or middle ar pressure is altered This mechanism is hought to be of less importance in isolated emporal bones as in our experiments

APPENDIX

As appears in Table II, the material comprises altogether 55 temporal bones 26 were from women, 27 from men In the case of two preparations it was not noted whether they were from a man or a woman, left or right, the age or whether they formed a pair 25 of the preparations were from the right side, 28 from the left 12 of the female preparations were from the right side and 14 from the left 13 of the male preparations were from the right side and 14 from the left There were altogether 19 pairs of temporal bones, 10 female and 9 male 15 of the preparations were not pairs, 6 right and 9 left 6 of the single preparations were female (2 right and 4 left) and 9 male (4 right and 5 left) The ages varied from 49 to 95 years The youngest woman was 49 years, the oldest 95 The youngest man was also 49, the oldest 88

Placing of tubes

Bulbus superior venae jugularis internae in 6 preparations, tegmen tympani in 48, through the ear canal together with a ventilation tube through the ear drum in 15 preparations, through the ear canal together with myringo-

tomia in 10 preparations, through the apex processus mastoidei in 2 preparations, through tegmen tympani and apex processus mastoidei in 1 preparation

Where the results of measurement have been omitted in Table II, some technical fault or other has arisen, e g calibration difficulties, leakage or poor contrast filling, such that the results of the measurement have been erroneous X-ray examinations were carried out under fluoroscope supervision (16 mA, 45–55 kV) Use was made of antero posterior, axialpyramidal, lateral and basal-axial projections for every preparation 26 preparations were in addition tomographed to localize possible cells not properly filled by contrast fluid For this purpose a Philips Polytome 100 with a hypocycloid movement was employed (2 mm cuts, 6 sec, 35 kV, 15 mA), with possible enlargement The examination by X-ray of the preparations when filled with fluid often revealed a small bubble of air under the inner opening of the tube, but this was so small that it obviously had no significance in connection with the total volume No cases of leakage of contrast fluid to the inner ear were etected

Fig 8 shows a preparation (no 41 in Table II) where X-ray examination after filling with fluid shows so much air that we felt unable to use the results determined by the fluid method

ACKNOWLEDGEMENT

We are greatly indebted to Mr Øyvind Lervik for invaluable technical assistance and to Miss Ellinor Kvam for doing all the writing

RESUMÉ

Le volume total de la caisse du tympan et des cavites mastoidiennes a été mesuré sur 55 os temporaux avec des membranes du tympan normales et pratiqué sur une serie d autopsie norvegienne Les mesures ont ete faites à l aide d une méthode acoustique et controllees par remplis de liquide après pompage à vide Un moyen de cont radiologique a été utilisé permettant le contrôle ra logique du remplissage Les volumes variaient de 2 à cm³ avec une moyenne se situant autour de 6 5 cm³

ZUSAMMENFASSUNG

Die Untersuchung umfaßt 55 Schlafenbeine mit norm Trommelfell aus einem norwegischen Sektionsma bei denen das Gesamtvolumen von Mittelohr und H raumen des Warzenfortsatzes gemessen wurde | Messungen erfolgten mittels einer akustischen Meth Diese wurde kontrolliert indem die zu untersuchen Hohlraume vollstandig entleert (Vakuum) und mit e Flussigkeit aufgefullt wurden Als Meßflussigke t w Rontgenkontrast verwendet so daß der Fullung rontgenologisch kontrolliert werden konnte Dieses V men betrug von 2–22 cm³ mit einem Durchschnitt ca 6 5 cm³

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A CLINICAL PILOT STUDY ON PREFORMED AUTOLOGOUS OSSICLES I

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stract The study is part of a project aimed at obtaining autologous preformed transplant for reconstruction of efect ossicular chain In animal experiments we found t bone could be produced in a titanium mould placed the tibia of the rabbit and dog To find out whether s observation was applicable in man 5 patients with sicular defects were selected A titanium mould con ning two chambers was placed in the proximal tibial taphysis for 6 months When the moulds were ex icted bone was found in 7 of the 10 chambers In 2 these the bone was stable and suitable for ossiculo isty On histological examination the same picture as the animal experiments was found an outer layer of rtical bone surrounding a system of spongy bone with arrow and haematopoiet c cells Microradiological exa ination verified the presence of mineralized bone t ssue

Most authorities are agreed that in clinical irgery the best bone for use as grafting aterial is that obtained from the patient s wn tissue ' (Burwell 1969)

Up until the 1950s surgery for chronic otitis nedia was chiefly adapted to meet the needs f pathology In 1952 Wullstein and Zollner resented their first works on modern tympa ioplasty Later the introduction of the intact canal wall technique by Jansen (1968) made it possible to obtain a middle ear of normal width ind also to reconstruct the ossicular chain in a iew way

There are two major problems connected with reconstructive ear surgery to create an ntact tympanic membrane and to achieve a sound conducting mechanism between the drum and the inner ear It is interesting to compare the evolution in myringoplasty with that concerning ossicular chain surgery

Hall (1951) used rice paper to cover an ear-drum perforation Wullstein (1952) and Zollner (1952) suggested postauricular skin grafts House (1960) described a technique using ear canal skin The results of these trials were initially good but the various techniques all had drawbacks More promising results were reported by Shea (1960) who used a vein graft In 1964 Ortegren presented a study into the possibility of using autologous temporal fascia to repair a drum perforation and reported good results Sheehy & Glasscock (1967) also re ported excellent results with this type of graft This material is easy to obtain and to handle and gives a high take rate regardless of the surgical technique used Fascia seems to be the tissue of choice for most surgeons today The choice of material used in myringoplasty has thus reached a stage where very few changes are called upon In surgery of the ossicular chain the development has how ever, been quite different There is no general agreement as to the best material for ossicular prostheses in spite of the many trials pub lished during the last two decades Wullstein (1955), Shea (1960), Austin (1965) and Shea (1974) presented studies with non biological implants Studies presenting results with ho-

mologous ossicles have been published by Austin (1976), Smyth (1975), Sheehy (1975) and Palva (1976) Jansen (1972) and Smyth (1975) advocated homologous nasal septum cartilage for ossicular chain reconstruction Autologous ossicles were used by Austin (1965), whereas Farrior (1960) tried cancellous bone and Ekvall (1973) used autologous cortical bone for reconstructive work

The fact that experienced otologists concerned with the problems of ossiculoplasty have such diverging opinions on the material indicates that the ideal prosthesis has not yet been found Some of the problems of getting a reconstructed ossicular chain to work in the long run are connected with resorption, bony fixation, extrusion, foreign body reaction etc To avoid such problems the ideal prosthesis ought to be a fresh autologous ossicle with an intact vascular system and surrounded by a protective membrane The idea of producing such a prosthesis came through an observation made in some animal experiments concerning integration of titanium screws in bone tissue (Brånemark et al , 1969) In a small hole in a titanium screw there was a tendency for growth of bone tissue to occur This led to animal experiments presented in a previous paper (Hallén et al , 1976) In that study we found that bone could be produced in a titanium mould placed in the proximal tibial metaphysis of dogs

To determine whether these experimental findings were applicable in man, the present clinical pilot study was carried out

The aims of the study were

1 to find out whether bone is produced in a titanium mould placed in the proximal metaphysis of the tibia in man

2 to investigate whether bone produced in this way has biological properties that would make it suitable for use as an ossicle in the middle ear, and

3 to test whether such an ossicle could be handled and used as a prosthesis in ossiculoplasty

It is not within the scope of this study to draw any definite conclusions concerning the lasting value of this new type of ossicular graft This can only be appraised after a long period of observation in several patients with meticulous follow up

MATERIAL

The patients were selected from the waiting list for ear surgery at the ENT Department, Sahlgren's Hospital, Goteborg Sweden according to the following criteria

1 Age between 20 and 50

2 Known or strongly suspected ossicular damage

3 A dry ear without discharge for at least one year

4 No signs of osteitis or cholesteatoma

5 A good speech discrimination score

6 Normal hearing in the contralateral ear

7 No general disease

8 A normal radiological examination of the proximal tibial metaphysis

Five patients were selected and pertinent clinical data for each patient are presented in Table I

All patients were carefully informed about the possible advantages and also about the risks with this type of surgery They were also informed that the procedure was part of an experimental clinical investigation The study was approved by the Ethical Committee for Clinical Trials of the University of Göteborg Sweden

METHOD

Five moulds were constructed out of titanium (ATi 24 Avesta Jernverk Sweden) (Fig 1) As in all reconstructive ear surgery the exact location of the ossicular defect was hard to predict even in these cases For this reason each mould was made with two chambers one of them designed for the situation with an intact stapes and the other one for those cases in which only a foot plate remained (Fig 2) The shape and size of the preformed ossicle

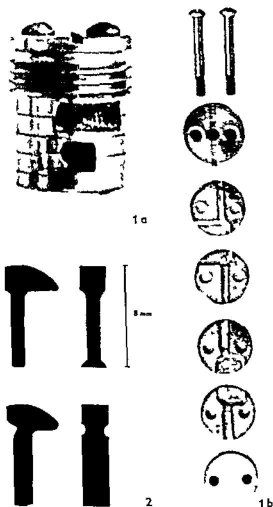


Fig 1 (a–b) The titanium mould assembled and taken apart
Fig 2 Shape and size of the chambers in the mould *Top figure meant for situation with just the foot plate* left Bottom figure meant for situation with an intact stapes

were based on the results found in an earlier study on the dimensions of the middle ear (Tjellstrom 1977) The moulds were made with a high degree of precision Their surfaces were carefully polished with ground pumice stone and then washed in tap water With the mould taken apart, it was then cleaned in five different baths of water under ultrasonic irradiation treated with trichlorethylene in order to remove all fat and finally washed in absolute alcohol After this cleaning procedure, the mould, still not assembled, was autoclaved on a titanium tray

Surgical procedure I

Under strict aseptic conditions the mould was placed in the left tibial metaphysis of the patient Under local anaesthesia with 5 cc of 2% Xylocaine with epinephrine 1 100000, a skin incision was made over the medial–anterior surface at the level of the tuberositas tibiae A periosteal flap was lifted up and a hole was made in the cortical bone with a specially constructed trephine burr of the same diameter as the mould These procedures were performed under generous saline irrigation to diminish heat trauma The wall of the hole was then threaded with a tap Some marrow was taken out and put into the chamber of the mould in the hope of facilitating osteogenesis The mould was then placed in the threaded hole of the bone and secured with a titanium screw driver This was also done under saline cooling The periosteal flap was put back in place and adapted with catgut sutures Haemostasis was established and the skin was sutured To minimize the surgical trauma as much as possible, all surgical work was done very cautiously A firm bandage was applied to the proximal tibia for one week after which the wound was inspected The sutures were removed 12 days after surgery The patients stayed at the hospital for 3 hours after the operation but were then allowed to move about and walk freely

Surgical procedure II

The second operation was planned according to the following schedule Six months after the mould was placed in the tibia the patient was admitted to the hospital for the ossiculoplasty The operation was performed under general anaesthesia and the middle ear was exposed through the postauricular approach The titanium mould was taken out from the tibia with specially designed instruments In those

Table I *Preoperative situation of the patients unfilled=defect*

Patient	Sex	Age	Diseased ear	Drum	Ossicular defect	Pure tone audiogram	Speech dcr score	A/B gap
S L	♀	43	sin	normal		43 dB	88 %	25 dB
K O	♂	41	dx	atrofic but intact		56 dB	100 %	20 dB
J O	♀	31	sin	dry central perf		50 dB	100 %	37 dB
K R	♂	46	sin	normal		78 dB	92 %	33 dB
H K	♂	24	dx	normal		45 dB	100 %	45 dB

instances in which bone suitable for ossiculoplasty was found, it was used for the reconstruction Bone tissue not used in the surgical procedure was put into a transport medium for later histological studies All handling of the bone was done in a glass container with the patient's own plasma In those cases in which no usable bone was found in the mould a prosthesis of cortical bone was made

During the surgical procedure a piece of cor- bone was taken out from the mastoid ..s and trimmed to form an ossicle with a mond burr under saline cooling in accordance with the usual routines for ossiculoplasties at our department This bone was then treated and studied in the same way as the preformed one In patients with a perforation of the tympanic membrane autologous temporal fascia was used as a drum graft underlay technique Gelfoam® soaked in saline solution was put into the middle ear to support it No Gelfoam® was allowed to come into contact with the new ossicle The ear canal was filled with surgical rayon and pieces of acrylic cotton The incision was closed with interrupted Polydec® sutures One week after surgery the ear canal packing was removed

RESULTS

The findings in each patient are presented separately below and a schematic drawing of the amount of bone found in each mould presented in Table II

Patient S L In the wide part of the moul bone was found but the shaft consisted on of a thin string of fibrous tissue The bo produced had a cortical layer surrounded l fibroblasts and a marrow cavity in the centr Because of the fibrous shafts, these bor could not be used for the ossiculoplasty I stead, a piece of cortical bone from the ma toid was used as a connection between th foot plate and the handle of the malleus

Patient K O Bone was found in the mo superficially situated chamber but not in th one in the marrow cavity of the tibia (Fig 3

Table II *Schematic drawing of the amount o bone produced in the different patients u filled=defect*

Patient	Chamber closest to the cortical endosteum	Chamber in the marrow cavity
S L		
K O		
J O		
K R		
H K		



Fig 3 A preformed autologous ossicle

middle of the shaft (Table II) Also in this patient the wider chamber had been placed most superficially

Patient H K No bone was found in either of the chambers They were filled with marrow surrounded by a fibrous tissue layer In this case a piece of cortical bone was placed between the foot-plate and the handle of the malleus

Four of the patients complained of pain from the implantation for some days and one of them had difficulty in walking freely for one week These symptoms were not expected After extraction of the moulds there was less discomfort No signs of local infection were found after any of the surgical procedures

In conclusion, bone was found in seven of the ten chambers However, in only two of them had bone suitable for ossiculoplasty been formed As shown in Table II, bone formation was more often observed in the superficial chambers than in the deeper ones It should be noted, however, that the narrowest chambers were always placed deepest in the tibia

Macroscopically, the ossicle had a cortical layer around spongy bone with marrow It was stable and easy to handle in the reconstruction In the other chamber no bone was found, only fibrous tissue

Patient J O In this patient the same kind of bone was found as in the patient S L A cortical shell of bone round a marrow space was produced in the wide ends of both chambers but in the narrow shafts only fibrous strings were found It was not stable enough to be used for the ossiculoplasty A malleus–stapes assembly as described by Austin & Glasscock was performed

Patient K R Both chambers contained bone of the same appearance as in the other patients However, in the chamber for the slimmest prosthesis there was a defect in the



Fig 4 Histological appearance of the bone produced Bone trabeculaes with filled osteocyte lacunaes surrounding a haematopoietic marrow An outer coating of mature connective tissue could be identified ×150



Fig 5 Microcardiogram of a preformed autologous oss cle An outer shell of mineralized bone t ssue could be ident fed

HISTOLOGY

The preformed osseous tissue that was not used in the middle ear of the patient was stud ied histologically and by microradiography

The chamber contained bone trabeculae surrounding a haemopoietic marrow with some fat cells On the outer side of the bone there was a 50–100 μm wide zone of mature connective tissue The bone trabeculae had val shaped filled osteocyte lacunae In some es there were osteoblast seams (Fig 4)

icroradiological analysis shows an outer ell of mineralized trabecular bone tissue (Fig 5)

DISCUSSION

In three out of the ten chambers no bone formation was detected only blood and fi brous tissue being identified In the remaining seven chambers bone tissue was found in two cases completely and in five cases incom pletely filling the chambers All bone produced had the same histological structure with an outer cortical layer and a central part consist ing of cancellous bone and red bone marrow On the surface of the bony cortex a layer of fibroblasts could be seen In nly two of the chambers was bone found in the entire shafts Thus it seems most difficul t l t un a for mation of stable bone in the n west part of the moulds It was also fou d hat the part of the chamber where least bone was pro duced was at the same time the most centr part of the mould with the longest distance to openings of the chambers Little is know about osteogenesis under these special cond tions but one possible explanation for the re sults might be that the chambers were to narrow

An interesting finding was that more bor seemed to be formed in the chamber that was placed closest to the endosteal surface of th tibia In animal experiments we were not ab to find any differences in the capacity for bone formation when the moulds were placed deep in the marrow cavity close to the endosteum or in the bony cortex (Hallén et al 1976) However it is known that the highest osteo genic activity is in the periosteal and the endo steal areas It is also known that there are differences between species concerning osteo genic capacity, which might explain the di fering results The level of the mould in the tibia could be changed to overcome this si ua tion At the insertion the moulds were fill with red bone marrow and marrow haema toma The demand on the body s capacity to form new bone tissue might however be to great To have the body remodel cortical bon might be a better method than the procedur used in this study Further studies will sho whether or not this is true Another factor that might be of importance is the time elaps between implantation and extraction of the moulds The time interval in the present stud was 6 months based on the results from ou animal experiments Perhaps this time was to long or too short for humans The stnre of fibrous tissue in the shaft observed in some patients did not give any definite informatio as to whether there had originally been bone tissue which had undergone resorption The lack of functional stress might have caused re sorption of the bony tissue

The present investigation has shown

(*a*) that under certain conditions the hum body can form bone tissue in a titanium mod placed in the proximal metaphysis of the tib

(*b*) that the macroscopic and microscopic ppearance of bone produced in this way sug est that it may possess properties that ought o be of great value for use as a prosthesis n tympanoplasty, and

(*c*) that bone tissue of this type can be andled at operation without detracting from ts major advantages

There is however, still much to be learnt about bone regeneration under special condi ions The results presented here indicate that it might be possible for the body to produce a preformed ossicle Based on the present find ings, the design of the next clinical pilot study will be modified

ZUSAMMENFASSUNG

Im Rahmen e nes Forschungsplanes versuchen wir ein autologes praformiertes Transplantat fur Rekonstruktion einer defekten ossikularen Kette zu erreichen In Tier experimenten haben wir zeigen konnen daß Bein in einer Titaniumform d e man in das Schienbein eines Kanin chens oder eines Hundes einbettet gebildet werden kann In der Absicht feststellen zu konnen ob diese Beobach tung auch fur Menschen gultig ist wurden fur d e Un tersuchung funf Patienten mit Gehorknochelchendefekten ausgewahlt In die linke proximale Tibiametaphyse wurde eine Form aus Titanium mit zwei Kammern fur sechs Monate eingebaut Als die Formen extrahiert wur den wurden Knochengewebe in sieben von den zehn Kammern gefunden Zwei Kammern waren mit Knochen gewebe daß sich fur Ossikuloplastik eignete ganz aus gefullt Die histologische Untersuchung ergab das gleiche Bild wie bei den Tierversuchen ein Netzwerk von spon giosem Bein m t Mark und blutbildenden Zellen von kortikalem Bein umgeben Die nachfolgende mikroradio logische Untersuchung bestatigte daß es sich um mi neral s ertes Knochengewebe handelte

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RELIABILITY OF BEKESY THRESHOLD TRACING IN IDENTIFICATION OF CARRIERS OF GENES FOR AN X-LINKED DISEASE WITH DEAFNESS

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Abstract Seven identified carriers and 20 potential car [illegible]

hearing impairment The sensitivity of the method is poor The specificity of the Békésy threshold tracing is high meaning that an absent dip cannot exclude the possibility of a subject being a carrier whereas a present dip can be regarded as an indication of a carrier When comparing conventional octave audiometry and Békésy threshold [illegible]cing the latter method is found to be the more subtle in [illegible]ing carriers of genes for recessive deafness There[illegible] Békésy threshold tracing may be of help in the ge[illegible]tic counselling of potential carriers of genes for recessive deafness

In epidemiological investigations it is a common finding that genetic factors account for hearing impairment in 50% of the cases (Barr et al, 1973, Fraser, 1974, Nance & Sweeney, 1975) The mode of transmission may be autosomal dominant, autosomal recessive, or sex-linked recessive It has been estimated that approximately 75–80% of genetic hearing loss is caused by autosomal recessive genes, dominant and sex linked genes accounting for the rest (Nance & Sweeney 1975)

Genetic hearing losses may be congenital or may develop during childhood or early or late adulthood (Konigsmark 1971) They may also be associated with abnormalities in other organ systems (Konigsmark 1969) An example of this is seen in Norrie's disease, also called progressive oculo acoustico cerebral dysplasia This disease has formerly been described in ophthalmological (Warburg 197[illegible]) and in audiological (Parving & Warburg 197[illegible]) literature The disease is well known as an X linked recessive trait, i e only affecting males with completely unaffected female carriers The penetrance is complete, so that a carrier has a 50% risk of having a carrier daughter or an affected son

Carriers of genes for recessive deafness cannot be identified by conventional pure tone audiometry (Wildervanck, 1957, Fraser, 196[illegible]) Carriers of X-linked recessive inherited hearing impairment may evidence minor audiological abnormalities (Nance, 1971) In 1968 and again in 1976, Anderson & Wedenberg reported that normal hearing carriers of genes for deafness could be identified by means of Békésy audiometry Among heterozygote carriers of genes for deafness, they found that 30% had small but distinct, peculiar "dips" in their Békésy audiograms

In an attempt to evaluate the reliability of the Békésy tracing method in identifying carriers of genes for X linked recessive hearing impairment, an investigation was performed on (1) normal hearing female subjects [illegible]

This work was supported by the Committee for Prevention of Blindness and Grosserer A V Lykfeldt og Hustrus Legat

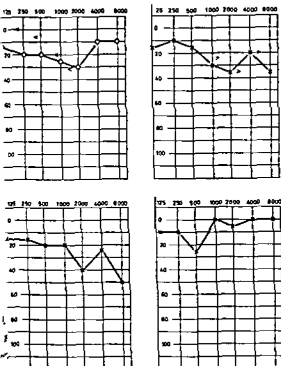


Fig. 1. Dip found by conventional pure tone octave audiometry in 3 known carriers and 1 potential carrier.

known female carriers of Norrie's disease, and 3) potential female carriers of Norrie's disease.

MATERIAL AND METHOD

Twenty nine female members of five families in which Norries's disease occurred could be found. For various reasons 2 of them refused to participate in the investigation. The remaining 27 women ranged in age from 11 to 69 years, average 36.4 years. Being a mother or mother's mother to affected boys, 7 of these were identified to be carriers of the disease. Twenty subjects were potential carriers. Eight members of the latter group were sisters to an affected brother. Five women were sisters of mothers to affected sons (aunts) and 5 were cousins through their mother's sister. Two were sisters of a mother's mother. None of the sisters or cousins had affected sons.

Otoscopy and pure tone octave audiometry were performed in all subjects. Thereafter, Bekesy audiometry was performed by means of a Bekesy accessory unit coupled to a Peters audiometer type Ap/6. The subjects were tested with both continuous and interrupted signals. In all tests the rate of attenuation change was 2.5 dB/sec. This attenuation rate was chosen because it gives no significant difference in excursion size with changing frequency (Siegenthaler, 1976). The signal changed the frequency at a speed control of 1/2 octave/minute. However, 3 subjects were examined with a changing frequency rate of 1 octave/minute due to age and discomfort in the sound proof hearing chamber. Two girls, aged 11 and 12 years, were not able to understand the Bekesy threshold tracing procedure. As the normal excursion size of Bekesy threshold tracing is 5–15 dB, the following criteria were used for the presence of "dips". The dip should be at least 20 dB HL and extend over at least 1 octave. As exogenous agents most frequently cause a hearing impairment in the frequency range above 3 kHz, the interval between 125 and 3 kHz was analysed.

Besides the family members, Bekesy audiometry was performed in 20 normal hearing female subjects. Their ages ranged from 23–69 years (average 37 years).

RESULTS

All of the family members examined had normal otoscopy. The results of the pure tone audiometry showed 21 subjects had normal hearing. Six subjects had a hearing impairment. One of them had symmetrical hearing impairment in the high frequency area (sloping audiogram) due to age. One subject was monaurally deaf and had a sensorineural hearing loss of 60 dB HL in the left ear, which was probably due to a previous vascular catastrophy in the cochleae. Three known carriers and one potential carrier had audiograms showing a monaural hearing loss of 25–40 dB HL at only one or two frequencies, the 3 known carriers most pronounced at 2 kHz,

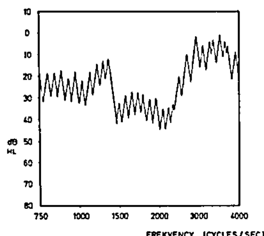


Fig 2 Typical dip seen in known carrier of Norrie s disease by Békésy threshold tracing Only continuous signal shown

and the potential carrier at 500 Hz (see Fig 1) No possible exogenous agent to account for this could be found

Among the normal hearing female control subjects, no dips fulfilling the aforementioned criteria were found by the Békesy tracing procedure Among known carriers, two had dips in the same ear centred around 2 kHz, where the peculiar dip was found by pure tone audiometry (see Fig 2) Five known carriers showed no dips in their Békésy threshold tracings, either by continuous or by interrupted sound stimulus Three potential carriers had dips, 2 sisters to affected brothers centered around 2 kHz, one in both ears, the other in only one ear In the last potential carrier the dip was centred at 500 Hz in the same ear as found by conventional audiometry All the remaining family members had no dips fulfilling the criteria (see Table I) The lack of agreement in the number between the conventional audiometry and the Bekésy threshold tracing in the known carrier group can only be explained by one subject s difficulties in understanding the Békésy tracing procedure Therefore, this subject had pure tone audiometry performed with the Peters Audiometer at frequencies surrounding the 2 kHz "dip"-centre frequency found by conventional audiometry The thresholds were determined by frequency intervals of 1 4 octave between 1 and 4 kHz This showed an increasing hearing loss from 1500 Hz through 1750 Hz up to 2 kHz and again decreasing through 2500 Hz and 3 kHz to nearly normal values at 4 kHz thus confirming the dip configuration This means that 42% of known carriers had dips in Békésy audiometry In 2 girls, aged 11 and 12, this modified "tracing procedure was performed to find a dip configuration centred around 2 kHz, but neither of them showed any tendency to dip configuration

DISCUSSION

If Békésy threshold tracing was theoretically the method of choice for finding all heterozygote carriers of genes for X linked recessive deafness, the following results from this investigation could be predicted Dips present in 100% of known carriers, and 50% among potential carriers, calculated on the basis of the mode of transmission However, in practice only 42% of known carriers and 15% of potential carriers showed dips" by Békésy threshold tracing This means that the method is not sensitive enough to reveal the majority of carriers This lack of sensitivity should be expected, as inherited recessive deafness is attributable to a great variety of different abnormal genes, and it would be difficult to imagine one single method being able to reveal all these abnormal genes

No dips were found in the normal hearing female control subjects In Anderson &

Table I *Results of pure tone octave audiometry and Bekesy threshold tracing in normals and carriers of Norrie's disease*

	Normal	Known carriers	Potential carriers
Number	20	7	20
Number of subjects with dips in Békésy tracing	0	3 (42%)	3 (15%)
Number of subjects with dips in the conventional audiometry	0	3 (42%)	1 (5%)

Wedenberg's control group of 133 subjects of both sexes, dips were present in only 2% of the normal ears This means that the specificity of Bekesy threshold tracing is at least 98% when regarding carriers of both autosomal and X linked recessive deafness Regarding specificity, our results are in agreement with Anderson & Wedenberg, who found 30% showing dips in a population of parents to children with autosomal recessive inherited hearing loss Therefore, it may be concluded that absence of "dip" in the Bekesy threshold tracing fulfilling the aforementioned criteria cannot exclude the possibility of the subject being a carrier In contrast, the presence of a dip is indicative of a carrier of recessive autosomal or X linked deafness Together with the family history, this may be of clinical importance

The small one sided endogenous "dips" found by conventional pure tone octave audiometry in 42% of the known carriers and in 5% of the potential carriers are in disagreement with former investigations of carriers of sex linked recessive deafness (Fraser, 1964, Nance 1971) Fraser reports that carriers of X linked recessive deafness have normal hearing In contrast Nance found in his investigation of stapedial foot plate fixation with perilymphatic gusher that all carriers of this X linked recessive hearing impairment had minor audiological abnormalities The controversy between these two reports may be due to the fact that the carriers examined were not carriers of the same disease

In this investigation the subjects were either 100% carriers or up to 50% risk carriers of genes for hearing impairment Only 42% of the known carriers showed a dip at pure tone audiometry This means that pure tone audiometry is not sensitive enough to exclude the possibility of the subject being a carrier of genes for hearing impairment If all possible exogenous agents can be excluded, the finding of a dip may be regarded as an indication of a carrier The dips may be specific for Norrie's disease

In the group of potential carriers, two sisters with dips were found at Bekesy threshold tracing The dip was not found by conventional octave audiometry This means that when comparing pure tone octave audiometry and Bekesy threshold tracing, the latter method is the more subtle in finding carriers of genes for recessive deafness However, investigations of a great number of known carriers of traits with known mode of transmission are ncessary to evaluate further this method

ACKNOWLEDGEMENT

I wish to express my gratitude to Mette Warburg M D head of the Copenhagen Eye Clinic for Mentally Retarded for referring patients with Norrie s disease

ZUSAMMENFASSUNG

Sieben identifizierte und 20 unidentifizierte Erbtrager von Norries Krankheit wurden mit Routine Audiometrie und Bekesy Audiometrie untersucht Die Untersuchung unterstutzt fruhere Befunde mit Bekésy Audiometrie auf heterozygoten Tragern von Genen rezessiver Schwerhorigheit Die Sensitivitat der Methode ist gering Die Spezifizitat der Bekesy Audiometrie ist groß dies bedeutet daß eine ausbleibende Besonderheit nicht die Moglichkeit ausschließen kann daß ein Patient ein Erbtrager ist wohingegen eine vorkommenden Besonderheit als Anzeichen eines Erbtragers angesehen werden kann Wenn man Routine-Audiometrie und Bekesy Audiometrie vergleicht findet man daß die Bekésy Audiometrie eine feiner reagierende Methode ist wenn Trager von Genen rezessiver Schwerhorigheit gefunden werden sollen Es wird gefolgert daß die Bekesy Audiometrie und die Familien-Anamnese zusammen helfen konnen wenn unidentifizierte Trager von Genen rezessiver Schwerhorigheit genetischen Rat ersuchen

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SUBJECTIVE DETECTION OF VERTICAL ACCELERATION A VELOCITY-DEPENDENT RESPONSE?

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Abstract Human subjective thresholds and directional sensitivity were investigated as a function of vertical linear acceleration with head erect A hyperbolic (r 0.94) relation emerged between threshold latency and acceleration magnitude (range 0.005 to 0.06 g) This implies that detection was determined by attainment of a given *velocity* (21.6+2.65 cm/sec) rather than the acceleration magnitude *per se* Re analysis of previous data from horizontal accelerations conducted with head erect and supine revealed similar hyperbolic relations (r 0.98 in both cases) with velocity constants of 22.6±1.28 and 32.4+1.96 cm/sec respectively From these findings it is inferred that with head erect (i e normal attitude re gravity) the thresholds to predominantly utricular (horizontal accel) and saccular (vert accel) stimulation were similar ($P>0.7$) However with head supine the saccular threshold was increased to approx 1.5×normal ($P<0.001$) The results also confirmed a previously reported difficulty in the subjective detection of the direction of vertical movement

Subjective orientation estimates have long been known to depend on the orientation of the head relative to gravity, with the constant feature that the *threshold* of sensitivity to gravito intertial stimulation tends to be *least* when the head is held in its normal erect position (Quix, 1925, Jongkees & Groen, 1946, Graybiel & Patterson, 1955, Graybiel & Clark, 1962, Schone, 1964) That this phenomenon is significantly dependent on the vestibular system is indicated by numerous observations For example, the general phenomenon persists after minimizing non vestibular somatosensory cues through water immersion (Schone, 1964) Again, there is substantial under estimation of apparent tilt angle in labyrinthine defective subjects exposed to changes of the gravito-intertial vector using the human centrifuge (Graybiel & Clark, 1965)

Since these phenomena occur in the absence of dynamic stimulation of the canals, it may be inferred that there is a significant tendency for the sensitivity of specifically the vestibular otolith organ to become progressively reduced as its orientation relative to gravity deviates from normal Indeed, for reasons detailed in the discussion, it seems that the phenomenon is only to be expected in the light of known structural and functional characteristics of that peripheral organ and its innervations

Taken together, these observations strongly suggest that for proper comparison of orthogonal (X, Y, Z) response characteristics, the head should be held in its normal erect, at

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Table 1 *Dependence of detectability of stimulus and directional assessment of accelera- upon stimulus acceleration magnitude*

Step acceleration magnitude *g*	0 005	0 007	0 009	0 011	0 015	0 020	0 040	[illegible]
% of undetected stimuli	28	17	13	13	3	0	0	0
% of wrong assessments of direction	30	28	38	26	29	23	34	[illegible]

titude relative to gravity Experimental results meeting this requirement are available for horizontal acceleration with head erect (Travis & Dodge, 1928, Lansberg, 1954, Meiry, 1965, Niven et al , 1966, Young et al 1966, Young & Meiry, 1968) However, although Z axis response has been studied during horizontal movement with the subject lying down (Jongkees & Groen, 1946, Walsh, 1961, 1962, Meiry, 1965, Niven et al , 1966), there is a dearth of modern data on the threshold response to vertical stimuli with head erect (Mach, 1875)

For this and other reasons mentioned below, Malcolm & Melvill Jones (1974) attempted to measure the subjective response of normally seated (i e head erect) human subjects to vertical accelerative movement using AE (Canadian National Aeronautical Es- ishment) computer controlled helicopter d later a NASA (Ames Research Center) vertical movement simulator Interpretation of their results was complicated by the unexpected finding that, in the absence of vision, subjects tended to become confused about the direction of movement which was not the case with Young & Mairy's subjects moving in a horizontal plane This difficulty seems the more surprising in view of the fact that good directional information is available in both primary afferent (Fernandez & Goldberg, 1976*a* and *b* monkey) and central (Daunton & Melvill Jones, 1973 cat) vestibular neural units responding to Z axis (body longitudinal axis) acceleration Furthermore associated studies of vertical eye movement (Melvill Jones et al 1976) and spinal motoneurone excitability (D Watt, personal communication 1977) during sinusoidal vertical movement revealed significant directional information in the observed patterns of these forms of in- voluntary reflex response

The present experiments set out (1) to meas- ure normal threshold sensitivity to vertical acceleration with head erect, (2) to compare these results with those of Young & Meiry conducted in a horizontal plane and (3) to investigate further the peculiar difficulty of directional assessment referred to above In addition, it transpired that, at least over the range of these experiments, the subject's threshold of movement could be identified with a constant time integral of the imposed acceleration, independently of the magnitude of the acceleration, suggesting that the threshold sensation was a velocity-dependent response

Similar methods to those of Young et al (1966) have been employed in order to facilitate comparison of results

METHODS

All experiments were conducted on the NASA Ames Height Control Test Apparatus (HCTA) referred to above Subjects were fixed to the seat of a blacked out cabin by a conventional aircraft restraining harness In addition a restraining headband was adjusted to maintain head orientation so that a line joining the infra- orbital margin and external auditory meatus was tilted downwards 30° relative to the horizontal to bring the major plane of the utricular macula close to the true horizontal The subject wore blackout goggles behind which he maintained open eyes The right hand was located on a light weight ... throw three position switch, the mid posi-

presenting zero response and the up and down positions signalling a subjective sensation of the direction of acceleration Ear muffs containing earphones permitted communication with the remote control cabin and attenuation of external auditory cues Provision was made to use white noise for masking troublesome external sounds but in practice this proved unnecessary

The additional head harness also served to avoid potential accessory cues from the canals due to pitching angular movements of the head potentially induced by the vertical linear accelerations with head tilted forwards The effectiveness of this restraint was verified by means of a small, sensitive, pitch detecting gyroscope mounted on a dental biteboard Test runs with 3 subjects exposed to the whole range of the experiment showed that angular head movements were usually undetectable and never exceeded ±0 5°

The main experimental series employed 8 adult subjects with no clinical abnormality One individual held a private pilot s licence but was not currently flying None of the others was an aviator They were each exposed on four occasions to each of eight magnitudes of step change in vertical acceleration Acceleration magnitudes were all very low, ranging from 0 005 g to 0 06 g as shown in the top row of Table I The distribution of acceleration magnitudes was chosen to concentrate recordings in that part of the data set where the most rapid change of response latency with acceleration was expected from the previous horizontal acceleration data of Meiry (1965) An 8×8 Latin square design permitted exclusion of learning effects and also a determination of whether practice during the experiment led to significant shortening of response latency

All subjects were practised over the range of the experiment and informed of their performance during these runs They were required to flick the indicator switch up or down as soon as possible after sensing the acceleration and according to the sensed direction of movement Practice was continued until subjects were satisfied they knew what to do

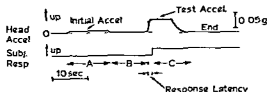


Fig 1 Records of stimulus (head acceleration) and response (subjective response from a single test run) The initial acceleration (A) was always 0 005 g either up or down The plateau velocity (B) was always 2 ft/sec either up or down In this example an upward acceleration of 0 04 g was imposed after 10 sec of plateau velocity and correctly identified with a latency of 1 1 sec

A potentially complicating factor was static friction of the cab in its track, which could produce a detectable jolt on commencing an acceleration from rest Consequently all test accelerations were begun at randomly chosen times after achieving a steady linear velocity of 2 ft/sec, which in turn was always attained by means of the lowest controllable linear acceleration, namely 0 005 g However, since cab movement was inevitably associated with some vibration, this procedure necessitated avoidance of a simple relation between *direction* of acceleration and any sensed increase or decrease in vibration This was achieved by balancing the occasions when a given direction of acceleration stimulus would be associated with increasing or decreasing vibration

Vertical acceleration was recorded from two sets of linear accelerometers, one installed on the cab and a two-dimensional linear accelerometer fixed firmly to the scalp The head mounting was arranged so that one degree of freedom paralleled the earth horizontal in a fore–aft direction when the head was tilted 30° downwards and forwards The orthogonal axis was aligned with the true vertical The system allowed remote checking of the correctness of head position before each run, as well as readjustment of head position in the head harness when this proved necessary after rests between runs Simultaneous recording of cab

and vertical head accelerometers showed that, there were no significant differences between the outputs of cab- and head mounted accelerometer systems Also recorded in parallel with these outputs was the subject's switch position and relevant system parameters such as the servo command voltage, safety limiting control outputs and actual cab position derived from a track potentiometer

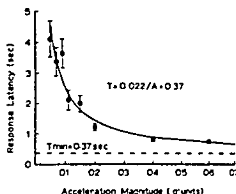


Fig 2 Dependence of response latency upon s.. acceleration magnitude The continuous curve sho.. calculated regression hyperbola which best fit these .. Brackets give standard error of the means (N=8 subj.. T=total response latency, A=step acceleration in g .. T_{min}=calculated asymptote for the present results

RESULTS

Threshold characteristics

Fig 1 represents an original record obtained from a single test run The upper trace is recorded from the vertically oriented linear accelerometer mounted on the head Starting from rest, there was an initial period of very low upward acceleration (A) at the standard value of 0 005 g (o 16 ft/sec²) until attainment of a steady, or plateau, upward velocity of 2 ft/sec Then, after a randomly chosen duration of between 4 and 10 sec (B) the test acceleration was applied (upwards in this example) and maintained (C) until after the subject had registered his response by flicking the switch up or down (up in this example) The response latency was assessed as the time between initiation of the recorded test acceleration and the registration of subjective response In practice all records were tape recorded and these latencies were measured from records played back on a suitably expanded time scale

Fig 2 shows the mean values of latency (±Standard Error) obtained in this way for all subjects and all runs at each of the eight acceleration magnitudes The values shown in this figure are independent of whether the subject made a correct or incorrect assessment of direction and, of course only those occasions when responses were indicated contribute to the curve In practice and as will be described below, all subjects responded on all possible occasions at acceleration magnitudes above 0 015 g However, as shown in the middle row of Table I, and as is to be expected progressively fewer test accelerations were detected as acceleration magnitudes decreased below this value

The curve drawn in Fig 2 shows the calculated least squares regression line fitted to the average latencies according to the hyperbolic relation

$$T=B/A+T_{min} \quad (\text{for } A>0\,005\ g)$$

where

T=mean measured response latency (sec) .. step acceleration magnitude (g), T_{min}=reaction time independent of A B=slope of the regression line when plotting T against $1/A$

The close fit of this calculated regression curve (r=0 94) implies that the value of B in eq (1) represents a meaningful constant The important feature of this conclusion is that this constant has the dimensions of linear velocity as is evident in the alternative form of the equation

$$B=(T-T_{min})A$$

From this observation the significant inference may be drawn that over the whole range of this experiment, the value B represents a consistent threshold *linear velocity* which had to be attained before generation

sensation of the changed movement The lculated value of this velocity (B) for the gression curve of Fig 2 is 0 022 g sec (=0 71 sec=21 6 cm/sec) Amongst the 8 subjects sted, the individual calculated values of B nged from 14 8 to 27 0 cm/sec (S D ±5 3)

T_{min} in eq (1) and (2) is interpreted as the nstant residual reaction time for initiation of echanical movement of the "up–down" lever ter perception of changed movement The lculated value of this residual reaction time, $_{min}$, is 0 37 sec This value is shown graphi ally as the dashed straight line in Fig 2, re resenting an asymptotic limit of the cal ulated hyperbolic regression curve

'omparison with previous ' horizontal" data

n order to compare these results with the horizontal" data of Young et al (1966), their esults have been re fitted with ' least quares" hyperbolic regression curves of the orm shown in Fig 2 In both instances, re narkably close fits were obtained, the cal ulated correlation coefficient, r being 0 98 or both their erect and supine conditions Also, as with the present results, meaningful values of T_{min} were obtained (0 7 and 0 6 sec respectively) although in both instances the actual values were somewhat longer than the corresponding value of 0 4 sec from our re sults

Graphical comparison of results is presented in Fig 3 Here the ordinate gives response times for subjective detection of acceleration *after subtraction of the respective minimum reaction time* (T_{min}) so that all curves begin at the same origin The abscissa gives the *inverse* of stimulus magnitude, expressed in non-dimensional units of g^{-1} Plotted in this way the hyperbolic relation of Fig 2 becomes a linear one in which, according to the expression in eq (2) the slope B numerically determines the constant velocity threshold sug gested above The figure shows calculated linear regression lines for the three sets of results The continuous line depicts the same line as that plotted in Fig 2 and represents the results from the present experiment The cor responding data from Young & Meiry's ex periments conducted in a horizontal plane with subjects seated erect and lying supine are shown as the dashed line and the dash dot dash lines respectively

The first notable feature is the near super-position of results for vertical and horizontal stimuli with the head held in its *normal orientation* relative to gravity The two lines are statistically indistinguishable ($P>0$ 7), the respective values of the coefficient B being 21 6 ± S E 2 65 and 22 6 ± S E 1 28 cm/sec The close conformity of the two data sets is particularly striking bearing in mind the fact that they were obtained from different experiments using different subjects, equipment and directions of acceleration It seems reasonable to conclude that, taken together, they represent the normal threshold of response to linear acceleration over the low amplitude range of these experiments for vertical (up–down) and horizontal (fore–aft) directions of movement

In marked contrast, the results from Young & Meiry's subjects exposed to horizontal ac celeration in a supine attitude yielded a con siderably higher value of B=32 4 ± S E 1 96 cm/sec for the ' velocity" constant ($P<0$ 001) In view of considerations mentioned in the introduction as well as additional evidence discussed below, it is inferred that this difference is primarily due to the 90° deflexion of the head s Z axis from the gravitation vertical

Directional sensitivity

The results in Fig 3 show that, with head erect, there was no significant difference in threshold sensitivity to low amplitude ac celeration in horizontal and vertical directions However, whereas the horizontally acceler ated subjects could effectively estimate the directional component of the stimulus, Table I shows that this was not so for our vertically accelerated subjects The upper row of the table gives the acceleration magnitudes em ployed in these experiments The middle row

shows that, as expected, the percentage of *failures* to detect the *presence* of an acceleration decreased rapidly to zero as the magnitude of acceleration increased However, the bottom row shows that the percentage of *incorrect* assessments of the *direction* of acceleration remained essentially constant at about 30%, and was therefore independent of acceleration magnitude over the entire range of experiment Thus, in line with the previous findings of Malcolm & Melvill Jones (1974) these results appear to demonstrate a specific difficulty in detecting the direction of vertically imposed movement with the head in its normal erect position

Additional observations

The statistical design of these experiments permits investigation of effects due to (*a*) practice, (*b*) up going versus down going accelerations and (*c*) increasing versus decreasing levels of vibration None of these influences produced statistically significant effects

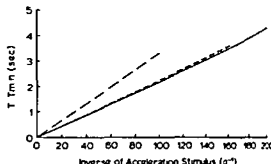


Fig 3 Comparison of results in Fig 2 (——) with th of Young Meiry & Li (1966) for horizontal accelera with head erect ( ) and in supine position (- after subtraction of the calculated minimum reac times (T_{min} in eq (1) and (2)) Note that response tim seconds (ordinate) is plotted against the *inverse* of acceleration stimulus (g^{-1}) The lines are calculated l squares regression lines for each data set with res tive correlation coefficients (*r*) of 0 94 0 98 and 0 95

DISCUSSION

hold sensitivity A velocity u response

unexpected feature of the results is the closeness with which the latency–amplitude plot of Fig 2 can be fitted by a hyperbolic curve relating response latency to the inverse of the imposed acceleration As pointed out in Results, this implies that over the whole experimental range the perception of movement was on average first sensed only after attainment of a fixed linear velocity *B* in eqs (1) and (2) The inference is substantiated by the additional finding that re analysis of previous results obtained in a similar way but different circumstances, by Young et al 1966 yielded equally close fits to hyp rela tionships There seems little doubt th these experiments it was the att nt o threshold velocity, rather than th ce ra tion *per se*, that determined the

tion of changed movement The finding closely akin to that associated with the w known "Mulder product (van Egmond et 1949) for the semicircular canals Thus dynamic characteristics of the semicircular nals can be approximated by an integrating gular accelerometer over a wide range brief acceleration stimuli, such that the p duct of acceleration and time to-detect i constant (1 5–2 0°/sec) However, neither rect measurement of otolith end-organ chanics (De Vries, 1950) nor system analysis of identified primary afferent sponse (Fernandez & Goldberg, 1976*b*) p to a peripheral vestibular origin for this defined integrative characteristic Analysis peripheral otolith units indicates the prese of a first order lag operator which could be sociated with the mechanics of otolithic m brane displacement However, the relev time constant determined by Fernandez Goldberg (median 16 ms for regular units a ms for irregular units) are more than two ders of magnitude too small to account for acceleration integration implied by the pres experiment Furthermore, their illustration regular and irregular unit responses to s duration force trapezoids (approximating

present acceleration profiles) show a rapid rise i unit activity following force onset, and no vidence of any long time constant integration f acceleration in the end organ

This raises the important question, to what xtent can the perceptual response to these ery low level accelerations be attributed to timulation of the vestibular sensory system? 'he matter was touched upon in the introduction, where studies were quoted in which subective sensations of change in direction of the erceived vertical were retained after minimisation of somatosensory cues by water immersion but were impaired in labyrinthine lefective subjects Furthermore, Young & Graybiel (unpublished) found that labyrinthine defective subjects had 8–15 times the normal threshold for detection of horizontal linear acceleration, which is similar to the earlier finding of Walsh (1961) Moreover, that the central neural vestibular response would be sufficiently sensitive to detect the low accelerations employed in these experiments is indicated by the very low thresholds (>0 005 *g*) reported for single acceleration dependent central neural units in the cat (Melvill Jones & Milsum, 1969, Daunton & Melvill Jones, 1973) Theoretical calculation of acceleration thresholds based upon the signal to noise ratio in the individual otolith nerve fibres are also consistent with the observed acceleration thresholds (Ormsby, 1974) Thus it seems that the very low acceleration amplitudes employed here, extending down to the absolute threshold of sensory perception (around 0 005 *g*, see Fig 2) would be unlikely to generate detectable bodily somatosensory cues but likely to activate suprathreshold response in the specially adapted vestibular organ

Implications concerning utricular and saccular components of response

Insofar as the above results can be ascribed primarily to vestibular stimulation, the close similarity between head erect responses to horizontal and vertical accelerations implies similar response characteristics in utricular and saccular components of the otolith organs Thus both the direction specificity of hair cell orientation (e g Spoendlin, 1966, Lindeman, 1969, Lowenstein & Wersäll 1959, Flock, 1964) and more recently the separate recording of primary afferent neurones from saccular and utricular maculae of the monkey (Fernandez et al, 1972, Fernandez & Goldberg, 1976*a*) show that, with head erect, horizontal and vertical movements predominently stimulate utricular and saccular end organs respectively

Additional results from the latter authors also have a direct bearing on the marked difference between threshold sensitivities determined in the direction of predominent saccular stimulation with the saccule in its normal (head erect) attitude and tilted 90° relative to this attitude (subject supine) The difference is seen by comparison of the continuous and the dash-dot dash lines in Fig 3 As stated in results, the respective slopes of these lines are 22 6 and 32 4 cm/sec, this difference being statistically highly significant From this finding it may be inferred that the threshold of sensitivity to saccular stimulation is significantly lower when in its normal orientation relative to gravity than when tilted 90° away from that orientation In the same context the above authors noted that saccular-dependent vestibular primary afferents responding to +Z (upwards re head) and − Z force vectors have similar steady state discharges with head erect, but the same populations have significantly different steady state values when the Z axis of the head is horizontal Assuming it is the differential firing rate between +Z and −Z units which constitutes the meaningful signal, then a given change of signal effected by a given acceleration would constitute a smaller proportion of the static differential signal with the Z axis horizontal than when in its usual vertical orientation According to Weber's principle the just noticeable-change (i e threshold) would then be associated with a larger stimulus when the Z axis was horizontal

Assume that the ratio of minimal detectable change in firing rate (Δf) is proportional to the spontaneous rate (f_0)

$$\frac{\Delta f}{f_0} = K$$

Assume further that the spontaneous firing rate for +Z saccular units (f_{0+}) is raised and that for −Z units (f_{0-}) reduced when the Z-axis is horizontal, and that the signal (μ) which must be detected is the difference between +Z (f_+) and −Z (f_-) unit rates, minus the difference in their spontaneous rates ($f_{0+} - f_{0-}$). The variances of the difference in signals (μ) is the sum of the variance of the +Z and −Z rates (f_+, f_-), which in turn are assumed proportional to their spontaneous rates. Consequently, an increase in f_{0+}, even though accompanied by a decrease in f_{0-}, will raise the standard deviation in the net signal, and increase the threshold for detection of accelera tion for Z-axis horizontal, relative to Z-axis vertical

Directional sensitivity

Turning to the question of our subjects' ap-[illegible] intensitivity to the direction of vertical [illegible], the possibility arises that they [illegible] responding simply to some form of [illegible] of vibration. A number of features, however, suggest this is an unlikely explanation. First, if the subjective response to acceleration carried good directional information, then since the sensory signal would presumably increase with increasing acceleration magnitude, one might expect there would then be a corresponding reduction of the directional uncertainty, which there was not. Moreover, similarity between the present data and those of Young et al (1966) over the range of experiment is too close to have been fortuitous and yet in marked contrast to the present re sults, Meiry's subjects who were exposed to *horizontal* acceleration were able to detect direction with confidence

Thus the characteristic of di[illegible]nal un certainty seems to have been as[illegible]ed specifically with the imposition of linear accele[illegible]tion in a vertical direction, parallel to gra[illegible]. This direction is the only one associated [illegible] no relative rotation of the gravito-inertial a[illegible]celeration vector. Possibly, therefore the a[illegible]sence of this variable is a contributory fa[illegible] (Benson & Bodin, 1966, Correia & G[illegible] 1966). Perhaps also the difficulty in percept[illegible] interpretation of the central message could [illegible] associated with a difference in the "need [illegible] know" about the direction of accelerat[illegible] horizontal and vertical directions. In this c[illegible]nection it is interesting to note that rec[illegible] results of Vidic et al (1976) have dem[illegible]strated differences between the oculom[illegible] reflex responses to accelerations stimulat[illegible] primarily saccular and utricular otolith e[illegible] organs, despite the similarity of subjec[illegible] sensitivity thresholds revealed in the pres[illegible] study

ACKNOWLEDGEMENTS

The expert technical assistance at NASA Ames Res[illegible] Center which made these experiments possible is [illegible]fully acknowledged as is the patient and co[illegible] contributions of our human subjects

ZUSAMMENFASSUNG

Subjektive Schwellen und Richtungsempf[illegible] vertikale lineare Beschleunigungen wurden in [illegible] Kopfhaltung untersucht. Zwischen Schwellen[illegible] Größe der Beschleunigung wurde eine hyperb[illegible] Beziehung (r=0 94) gefunden (im Bereich zwischen [illegible] und 0 06 g). Dies bedeutet daß die Schwelle eher [illegible] die absolute Geschwindigkeit (21 6±2 65 cm [illegible]) durch die Größe der Beschleunigung bestimmt [illegible]. Eine erneute Analyse von früheren Daten von ho[illegible]taler Beschleunigung mit aufrechter Kopfhaltung [illegible] Rückenlage ergab eine ähnliche hyperbolische [illegible]ziehung (r=0 98 in beiden Fällen) mit Geschwindigk[illegible] konstanten von jeweils 22 6±1.28 und 32 4±1 96 c[illegible]. Diese Ergebnisse zeigen daß bei aufrechter K[illegible]tung (also in der Normalposition bezüglich En[illegible]nigung) die Schwellen bei vorwiegender Stim[illegible] Utriculi (horizontale Beschleunigung) und Saccul[illegible] kale Beschleunigung) ähnlich sind (P>0 7). In R[illegible]lage ist die Sacculus Schwelle jedoch auf ung[illegible] über den Normalwert erhöht (P<0 001). Diese Er[illegible]te bestätigen die auch schon früher beschriebene [illegible]sicherheit im Erkennen der Richtung vert[illegible] [illegible]gungen

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LABYRINTHINE AND SOMATOSENSORY CONVERGENCE UPON VESTIBULO OCULAR UNITS

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Abstract The vestibular nuclei were investigated in 18 adult cats Vestibulo-oculo-motor neurons were identified by antidromic stimulation of the ascending medial longitudinal fasciculus (MLF) The neurons were subjected to various stimuli vestibular neck, forelimb and hindlimb nerve stimulation on both sides The recording was extracellular with micropipettes containing Fast Green Only the medial and the superior vestibular nuclei were found to project to the MLF All projecting units had input from the labyrinths Excitatory response latencies to ipsilateral labyrinth stimulation never exceeded 3 msec Both excitatory and inhibitory response latencies could be distributed into different categories The majority of the neurons did not receive a somatosensory input and surprisingly few convergent units could be seen Peripheral somatosensory information apparently plays a minor role in vestibulo-ocular relations

The labyrinth, vestibular nuclei (VNC) and secondary vestibular fibres to the nuclei of the extraocular muscles via the medial longitudinal fasciculus (MLF) play a major role in the control of conjugate eye movements

The eye movement control system moves the eye in order to maintain the image of the fixated object on the retina This image is displaced by the motion of the visual target and by changes in head position e g by rotation (Meiry, 1971) The vestibulo-ocular reflex stabilizes the position by generating compensatory eye movements to counteract movements of the head Bizzi et al (1972) have shown that this stabilization of the eye during head rotation is strongly influenced various feed back circuits Peripheral fe back would serve two important purpo It would compensate for output disturban that is, act as a regulator, e g for postu adjustments Secondly, it would act as a ser mechanism, e g for compensatory eye mo ments Both of these functions are utilized the control of movement and posture (H Henneman, 1974), in equilibrium control visual stabilization Thus, as a consequence a head movement, labyrinth receptors neck proprioceptors are activated and wi assistance of visual information, the re loops induce a compensatory eye move which permits the fovea to remain fixed relation to a point in visual space

However, Teixeira & Lackner (1976) ha observed that a change in the perceived p sition of the head in the absence of a physi deviation can influence eye posture This i cates that, under certain conditions the i ence of neck proprioceptor afferents is not rectly reflexive but must be centrally in preted before effecting oculo-motor co Evidence hinting that the area responsibl integrating the vestibular, proprioceptive visual information is the VNC, has rec been provided by Miles (1974), who f some VNC unitary activity to be corr with eye movements in certain situa These units may be involved in the prog ming of head movements (Zierau et al , 1

This project was supported by t e Cana Research Council (grant MA3311) and by Medical Research Council (grant 14X 4503)

This study was carried out to examine hether or not somatosensory input affects estibulo-ocular units and if so to investigate ie patterns of this input

MATERIALS AND METHODS

he series included 18 cats weighing 2–3 5 kg urgery was performed under halothane/ N_2O/O_2 anaesthesia Tracheostomy was done outinely Endtidal CO_2 was monitored with a eckman gas analyser and kept at 3 5–4 5% heating pad maintained body temperature at 6–37°C The anaesthesia was discontinued rior to the recording and the animal was given allamine triethiodide (Flaxedil) and artificial entilation was started Wounds and pressure oints were repeatedly infiltrated with Xylo- aine and precautions were taken to ensure hat the animal experienced no pain Through out the experiments there were no signs of iloerection or dilation of the pupils After the reparation the animal was placed in a David Kopff stereotaxic frame The body was sup- ported by a sling such that the limbs hung free

The electrode arrangement is illustrated by Fig 1 Stimulating bipolar silver electrodes Teflon coated except for the tip 125 μm in ulated diameter) were placed on the utricular ateral canal nerve on both sides via a ventro- ateral approach to the inner ear They were kept in place by low melting point (42°C) paraf in and fixed to the skull bone with acrylic ement Current spread to cochlear and facial nerves was controlled by using intensities ess than 3 5 times threshold (for evoked eye leviation) for vestibular nerve (VN) stimula ion (Rubin et al , 1977)

The radial nerves (RN) were dissected in oth forelimbs and placed on plastic rails ontaining bipolar stimulating stainless steel lectrodes The same procedure was repeated ilaterally for the sciatic nerves (SN) and the nerves to the cleidotrapezoid muscles (NN)

Through a craniotomy exposing relevant rain areas electrodes were stereotaxically laced on the ipsilateral (with respect to the recording electrode) and contralateral medial longitudinal fasciculi (MLF) The electrode consisted of two insulated stainless steel wires (100 μm uninsulated diameter, tapered tips 0 5 mm apart) in a glass cylinder with an exter nal diameter of 0 6 mm These electrodes were used for antidromic activation of VNC units Correct electrode positioning was determined on the basis of vestibular evoked potentials During repetitive stimulation of the ipsilateral VN the electrode was slowly lowered towards the MLF The point at which the largest field potential could be recorded was taken as the location of the MLF The average response latency was 1 7($\pm$0 2) msec After the record ing current was passed through the MLF elec trode to produce a lesion for histological loca lization Of course, the method employed does not provide proof of a selective stimulation of the MLF It is also likely that adjacent struc tures have been activated

Glass microelectrodes (2–4 MΩ 2 M KCl) saturated with Fast Green FCF (Thomas & Wilson 1965) were used for extracellular re cording in the VNC It was reached through a posterior cranio-laminectomy and the elec trode was angled so as to leave the cerebellum intact The correct electrode position in the VNC was achieved through stereotaxic cal culations and the method described by Shi mazu & Precht (1965) At various electrode positions dye was deposited electrophoreti cally to aid in the subsequent histological analysis Mechanical stability of the record ing system was obtained by covering the ex posed area with 3% agar gel

The microelectrode was connected to an a c amplification system A PDP8E com puter was employed for on line analysis of the recordings which also were stored on a Philips analog 7 tape recorder for off line analysis (Sigma V Computer) The microelectrode was lowered through the VNC while stimulating the ipsi and contralateral MLF respectively Neurons antidromically activated by MLF stimulation were regarded as projecting to the oculomotor nuclei Criteria for judging the ex

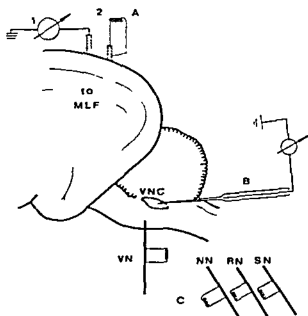


Fig 1 Arrangements of stimulating and recording electrodes *A* is the bipolar electrode which was lowered into the medial longitudinal fasciculus (MLF) A correct position was determined on basis of recorded evoked potentials using a peripheral vestibular stimulus (*1*) This electrode (2) was employed to antidromically activate neurons in the vestibular nuclear complex (VNC) Extracellular recording in the VNC took place (*B*) Peripheral stimulation consisted of single electric shocks administered through implanted bipolar electrodes in the vestibular nerve (*VN*) the radial nerve (*RN*) the sciatic nerve (*SN*) and the nerves to the cleidotrapezoid muscle NN) bilaterally

)

citation of a neuron as antidromic have been reviewed by Kelly et al (1975) and the specific problem of the collision test has been further analysed by Fuller & Schlag (1976) The interpretation of the antidromic response is in accordance with their opinions and results Once a MLF projecting VNC unit was identified its spontaneous activity, if any, was recorded It was then subjected to various stimuli, bilateral VN stimulation was followed by bilateral RN SN and NN stimulation

Peripheral nerve stimulus consisted of square waves (0 1 msec duration frequency 0 4–1 Hz) and the stimulus intensity was kept at 1 5 times threshold for evoking a muscle twitch prior to immobilization The evoked neuronal activity was amplified at a band width of 1–3 000 Hz to show field potential and unitary activity on the oscilloscope for photography Low frequency was cut off at 300 Hz for triggering standard pulses by spikes to be displayed in post stimulus time histograms (PSTH) Bin widths could be varied for 1X bins per histogram

The animals were killed with a lethal dose of pentobarbital and perfused with 10% formaldehyde in saline Serial 50 μm thick frozen sections of the brain stem were cut in the plane of the electrode series and stained according to Kluver Barrera Tracing of electrode sites in the MLF was done on 100 μm cresyl violet stained frozen sections The dye marks and the lesions aided in identifying recording positions The boundaries used to separate the individual vestibular nuclei were those described by Brodal & Pompeiano (1957) and Berman (1968)

RESULTS

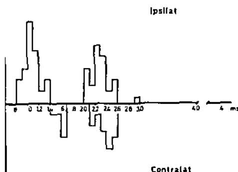
97 VNC neurons projecting to the ascending MLF (MLF units) were encountered Although all four major vestibular nuclei were extensively investigated MLF units were found to be confined to the superior and medial nuclei (SVN and MVN) The lateral and the descending vestibular nuclei thus contained no cells with antidromic responses to MLF stimulation This observation is based on an analysis of 344 neurons

25% of the projecting neurons were found in the SVN and the remaining 75% in the

Table I *Types of labyrinthine input for VNC neurons projecting to the MLF*

Figures indicate number of neurons

Stimulus	Response in MVN		Response in SVN	
	excitatory	inhibitory	excitatory	inhibitory
Ipsilat VN	57	15	12	11
Contralat VN	0	9	0	3
Ipsi and contra lat VN	16	5	9	4
Total	73	29	21	18



2 Response latencies for neurons in the medial vesti r nucleus All responses are excitatory Stimulus is as ie following figures single electric shocks to the VN and contralaterally

'N As more tracks passed through the re easily accessible MVN, positive con sions cannot be drawn regarding the per itage of projecting cells in different nuclei

byrinth input to MLF projecting MVN urons

l units in the MVN projecting rostrally sponded to VN electrical stimulation with an citation and the majority (78%, $n-73$) ere influenced solely by ipsilateral stimulus able I) The remaining 22% could be tivated from both labyrinths No neuron was und to increase its spontaneous activity hen only a contralateral stimulus was given round one third of the 73 MVN neurons ere also inhibited (Table I), many by contra teral labyrinthine input Fig 2 illustrates the sponse latencies of the analysed cells when N stimuli were given Those units having an icreased firing rate due to ipsilateral laby nth stimulation fell into two latency cate ories One group responded monosynapti ally (0 8–1 4 msec) The second one also howed a rapid activation (2 0–2 6 msec) ossibly including both di and trisynaptic re ponses At this latency range the demarca on between late disynaptic and early tri ynaptic responses becomes quite indistinct

Contralateral labyrinth inflow also caused wo latency groups (Fig 2) No monosynap c responses were observed although both groups had short latencies The first one was definitely within the disynaptic range (1 4–1 7 msec) and the second one closely paralleled the corresponding group in the ipsilateral population (2 1–2 6 msec)

The latencies for MVN neurons responding with an inhibition are illustrated in Fig 3 Due to the difficulty in determining onset of the inhibition, larger bin widths were employed than was the case for excitatory responses (0 5 vs 0 1 msec bins) The number of units in this sample is quite small (29) but it is still evident that ipsilateral VN stimulation gives rise to two distinct short latency groups (less than 10 msec) One population had a rapid inhibitory response, 2 5–4 0 msec indicating that although monosynaptic inhibition was absent, nevertheless few interneurons were involved in this inhibitory pathway A more complex neuronal system is indicated by the latency of the second population 7–8 5 msec

Even fewer units (9) were found which responded to contralateral labyrinth stimulus with inhibition However, on a first approximation it appears as if there are two latency populations which more or less correspond to the ones seen for ipsilateral input

Labyrinth input to MLF projecting SVN neurons

Of the 24 neurons recorded in the SVN, 21 were excited by VN stimulation and 15 of these were also inhibited The remaining 3 cells showed only an inhibitory response (Table I)

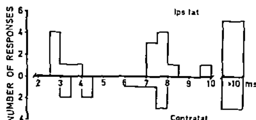


Fig 3 Inhibitory response latencies for neurons in the medial vestibular nucleus Ipsilat and contralat refer to ipsi and contralateral electrical VN stimulation

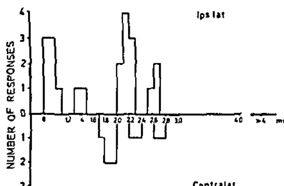


Fig 4 Excitatory response latencies for neurons in the superior vestibular nucleus Ipsilat and contralat refer to ipsi and contralateral electrical VN stimulation

As in MVN the majority (57%, $n=21$) of the excited units could only be activated from the ipsilateral labyrinth Again, no neurons were observed to be excited from the contralateral labyrinth Twice as many units in the SVN responded to bilateral labyrinth input as was the case for MVN The majority of the inhibited neurons (61%, $n=18$) were affected by ipsilateral labyrinth stimulus Approximately as many cells responded to contralateral as to bilateral stimulation

SVN units were also analysed in terms of eir excitatory response latencies which are ven in Fig 4 There is still the problem of the smallness of the sample, which allows more for suppositions than for statements With regard to ipsilateral inflow, one population demonstrates monosynaptic excitation, 0 8–1 5 msec, while the second one has late disynaptic and/or early trisynaptic response latencies, i e the same two groups as were observed in the MVN Contralateral VN stimulation evoked response latencies in the range 1 7–2 8 msec Thus the latency grouping of excitable neurons appears to be quite similar for MVN and SVN

Proportionately more units in the SVN than in the MVN were inhibited (Table I) Response latencies are illustrated in Fig 5 Although two distinct latency populations were observed when an ipsilateral stimulus was employed, we may still be dealing with a single one, considering the number of cells

Units with response latencies slower than msec were not analysed in terms of laten population grouping Contralateral labyrin inflow gave rise to a similar latency pattern was observed for the ipsilateral group (Fig The number of cells was smaller

Somatosensory input to MLF projecting units in the VNC

Twenty five neurons in the MVN and 9 in t SVN could be influenced by somatosenso volleys, not only evoked by electrical sing shocks to the dissected peripheral nerves b also due to manual stimulation of differe body parts, i e joint manipulation touchi of fur and skin and "deep' pressure Th 35% of the investigated neurons (n=3 demonstrated a convergence of labyrinthi and somatosensory input No specific patte with regard to side of labyrinthine activatio vestibular nucleus, or somatosensory rece tive fields could be delineated with certain but certain combinations of stimuli seemed predominate The MVN cells with ipsilate vestibular input were in 50% influenced neck stimuli ($n=18$) None was encounter responding to a neck and hindlimb or fo and hindlimb stimulus combination M cells were activated but some were inhib by the somatosensory stimulus as shown Fig 6 This neuron is located in the MV Ipsilateral VN stimulation causes a po

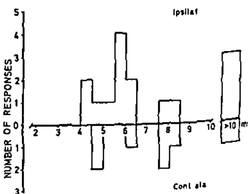
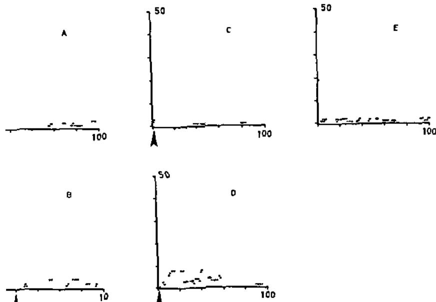


Fig 5 Inhibitory response latencies for neurons in t superior vestibular nucleus Ipsilat and contralat refer ipsi and contralateral electrical VN stimulation



6 Histograms illustrating the different responses of euron in the MVN to vestibular and somatosensory uli Number of spikes on the Y axis and time in msec the X axis Arrows indicate onset of stimulus *A* and how the post-excitatory inhibition to ipsilateral VN stimulus with different time scales 100 and 10 msec respectively (*C*) Inhibition due to ipsilateral NN stimulus (*D*) Excitation on contralateral RN stimulation *E* shows the spontaneous firing rate of the neuron

citatory inhibition, while ipsilateral NN mulation inhibits the spontaneous rate The ency is around 7 msec The observed tivation from the neck was usually shorter, 4 msec When the ipsilateral RN was imulated the result was an excitation with o bursts, the rapid one having a latency of msec

The somatosensory input to the seven MVN urons with bilateral vestibular inflow emed to arise from wide receptive fields in ck fore and hindlimb regions bilaterally

Neurons in the SVN responded to soma sensory stimulation in a similar manner as scribed for the MVN Receptive fields were quently located in neck and forelimb re ons and activation from hindlimb areas was ily seen when also neck and/or forelimb elds were involved The SVN neuron in Fig has no proven input from hindlimb but from ck and forelimb somatosensory receptors silateral labyrinthine stimulus produces a synaptic activation followed by an inhibi on and the responses evoked from the contralateral side show roughly the same charac teristics

DISCUSSION

MLF projecting units were restricted to the SVN and MVN This is in agreement with the anatomical findings of Tarlov (1972) who was unable to demonstrate direct vestibulo-oculomotor fibres arising in the lateral or descend ing vestibular nuclei McMasters et al (1966) claim to have found vestibulo-oculomotor projection from all four vestibular nuclei which however, is believed by Tarlov (1972) to be due to incidental damage to the MVN Studies by Stein & Carpenter (1967) and Gacek (1969) have corroborated the concept of MLF fibres arising only in the SVN and MVN

In the present study, it would appear that a larger proportion of SVN units projects to the MLF than in the MVN, when proper regard is taken to number of cells recorded and electrode tracks in the two nuclei This is not

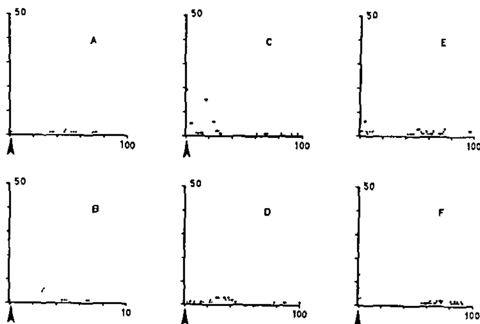


Fig 7 Histograms illustrating the different responses of a neuron in the SVN to vestibular and somatosensory stimuli Number of spikes on the Y axis and time in msec on the X axis Arrows indicate onset of stimulus *A* and *B* show the disynaptic excitation followed by an inhibition elicited from the ipsilateral labyrinth Time scales are different (*C*) Ipsilateral RN stimulus caused this excitatory–inhibitory response while contralateral RN contralateral NN or SN stimulation had no effect (*D*) Response to ipsilateral NN stimulus (*E*) Spontaneous firing rate of the neuron (*F*) shows the response to contralateral VN stimulus It has about the same characteristics as in *A* and *B* but the latency is about 1 msec longer

totally unexpected, since many MVN units also project to the cord and the cerebellum, whereas the SVN projection is predominantly directed towards the oculomotor complex (Brodal, 1972)

In the MVN, all MLF projecting units received an ipsilateral labyrinth input, while in the SVN 92% were of this type Furthermore, no unit could be excited from the ipsilateral labyrinth with a response latency shorter than 3 msec

This is logical since we are dealing with a system, often trisynaptic (Szentágothai 1964), which upon changes in head position must make almost instant compensatory eye movements in order to keep the fixated image stabilized on the retina

As seen for other projection systems (Rubin et al , 1977) inhibitory input to VNC units was present—though of considerably longer latency than excitatory input Both excitatory and inhibitory inflow seemed to be split into several latency groups Such a finding implies the existence of several different input path ways Besides the monosynaptic ipsilater labyrinth input to VNC neurons projecting t MLF there are disynaptic and polysynapt pathways In addition to the three neuron ar with a synapse in the VNC there are multi synaptic pathways which also connect th VNC to the eye muscles (Szentágothai 1964 Their importance is shown in the experiment by Shanzer (1964)

The MLF projecting VNC units were foun to be scarce in somatosensory input an consequently few showed labyrinth-soma sensory convergence Wilson et al (196 observed only 32% of the MLF units to facilitated by shocks strong enough to act vate group III fibres

Fredrickson et al (1966) described lab rinthine and somatosensory convergence for large proportion of analysed VNC neuro The somatosensory input was predominan in the form of proprioceptive information fro the neck and limbs, especially from proxim

ints According to Pompeiano & Barnes 971) it is highly improbable that Ia muscle ferents contribute position information di- ct to the VNC The present study only deals ith the VNC cells projecting to the MLF d here somatosensory and labyrinthine con ergence appears to be proportionately less xtensive than for the VNC cell population as whole However, when this convergence as observed there was a strong preference or input from receptive fields in the upper arts of the body, especially in the neck re ion Manual stimulation of the limbs in- icated that joint manipulation, above all roximal joints like hips, shoulders and el ows, was frequently the most effective imulus with regard to somatosensory activa- on of the neurons The majority of neurons eceived antagonistic input from the two sen- ory systems which is in line with earlier re- orts (Fredrickson et al, 1966)

Various possibilities may account for the arce somatosensory input reported, includ ig methodological factors However, when an dividual semicircular canal is stimulated, eye iovements are induced in the planes parallel the plane of that canal—regardless of the osition of the eyes and the orbit or of the ead and the neck (Cohen et al, 1966) Under iese circumstances there is little need for in ormation from receptors in the neck or other arts of the body

The stimulus was administered to the nerves om the lateral semicircular canal and the tricle The otolith organs compensate for atic head tilt by inducing counterrolling Dekleyn 1921) The neck receptors do not ontribute significantly to this type of eye iovement when the head is subjected to a atic tilt (Hannen et al, 1966, Cohen et al, 970) Dynamic compensatory counterrolling, n the other hand, can be larger—hence the eed for inflow from neck afferents

ZUSAMMENFASSUNG

ie Vestibulariskerne von 18 erwachsenen Katzen wur en untersucht Vestibulo-okulomotorische Neuronen wurden durch antidromische Stimulation des Fasciculus longitudinalis medialis (MLF) identifiziert Die Neuronen wurden verschiedenen Stimulus ausgesetzt vestibularen Hals Vorder und Hinterbein auf beiden Seiten Die Registrierung erfolgte extrazellular mit „fast green" ge füllten Mikropipetten Nur bei den medialen und superio- ren Vestibulariskernen wurde eine Projektion auf MLF nachgewiesen Alle projizierenden Neuronen hatten Afferenz von den Labyrinthen Die Latenzen für exzitie rende Antworten auf ipsilaterale Reize des ipsilateralen Labyrinthes waren nie langer als 3 msec Sowohl an regende als auch hemmende Antwortlatenzen konnten in verschiedene Gruppen getrennt werden Die Mehrzahl der Neuronen erhielt keine somatosensorische Afferenz und in Übereinstimmung damit fanden wir nur wenige konvergente Neuronen Periphere somatosensorische In- formation spielt offenbar eine geringe Rolle für die vesti- bulo-okularen Verbindungen

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INHIBITORY AND PROMOTING EFFECTS OF SUBLIMINAL PENDULAR ROTATION ON OPTOKINETIC NYSTAGMUS IN MAN

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(Received March 17, 1977)

stract The influence of vestibular stimulation by sub-iinal pendular rotation on the optokinetic nystagmus in rmal human subjects is discussed Cortical OKN with ndular rotation was promoted in three indexes—eye eed frequency and total amplitude The subcortical KN increased markedly when the slow phase of OKN d the compensatory eye movement resulting from the ndular rotation were in the same direction However, was inhibited when the two directions were mutually iposed From the results obtained in the present experi ent and in the previous study by the author, it can be oncluded that the vestibular organs effect on visual func on is not only promotive but also inhibitive

is a well known fact that there is a common entral pathway and an integration between ie vestibular system and the optokinetic sys-'m The present experiment was performed sing normal human subjects, in order to study ie mechanisms between the two systems

MATERIALS AND APPARATUS

'he subjects were 20 healthy students, aged 9–23 years Fig 1 shows a small optokinetic cylinder, 10 cm in diameter, 10 cm in height, and 2 kg in weight, having 8 black stripes 1 cm in width on its surface The cylinder was rotated at a constant speed of 200°/sec A con vex lens of 4 D was placed between the eyes and the cylinder to enlarge the visual field The optokinetic stimulation was applied to the subjects according to the method of Jung (1953), who described the problem of the in-tention of the subject in the optokinetic nys-tagmus (OKN) test Namely, when the sub-jects gaze attentively with interest at the stripes on the cylinder, the cortical form of OKN is induced, and when looking unatten-tively at the cylinder, the subcortical form of OKN is induced

A rotatory chair of Bárany's type was used for the pendular rotation The chair was ro-tated manually under conditions of a pendular rotatory angle of 90° for a period of about 6 sec Under these conditions no nystagmus

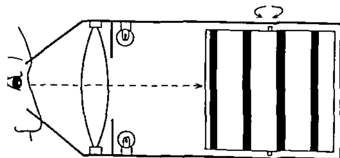


Fig 1 The optokinetic cylinder

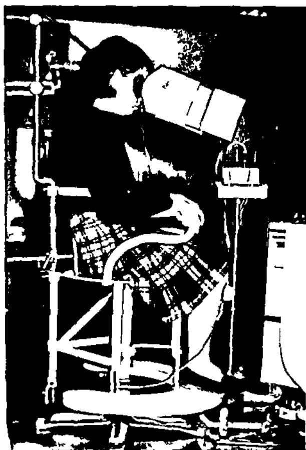


Fig 2 The optokinetic cylinder fixed on the rotatory chair

as observed with eyes closed—or with the eyes open—in the dark

The subjects were tested sitting in the chair to which was fixed the optokinetic cylinder (Fig 2), in this way they were subjected simultaneously to pendular rotatory stimulation and to optokinetic stimulation The nystagmographic recording of horizontal eye movements was done using time constants of 2 0 sec and 0 03 sec and a paper speed at 1 cm/sec or 10 cm/sec

METHODS AND RESULTS

Experiment 1

First, 20 subjects were tested by subjectr them to optokinetic stimulation alone In th experiment, the subjects were ordered to g attentively at the movement of the stripes the cylinder After an interval of about 10 m the next optokinetic test was done with t subliminal pendular rotatory stimulation T OKN was recorded for a period of 10 sec af the start of the optokinetic stimulation and t frequency and the total amplitude were ca culated, and then the maximum eye speed the slow phase for the next period of 10 se The mean values of R OKN and L-OKN we used The absolute values of both the ampl tude and the eye speed were calibrated fr the values obtained with an eye movement be tween two visual points 10° apart

A case of the recorded wave of OKN w out and with pendular rotation is shown Fig 3 The values of OKN obtained are show in Table I Table II also demonstrates stat tically, by using the table *t* , that each val of OKN increased significantly together w the pendular rotation The increase in e speed was most remarkable

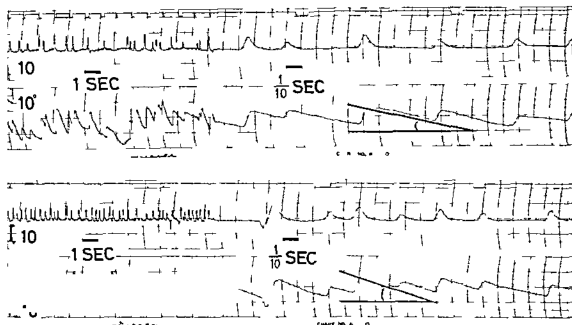
Experiment 2

Five subjects in all were tested in the ne study, in order to observe the influence the subliminal rotatory stimulation on the su cortical OKN At that time, the subjects we ordered to look unattentively at the cylinder

As shown in Fig 4 the increase in and the i hibition of OKN were observed in connectio with a period of pendular rotation The wav

Table I *Values of cortical OKN without and with pendular rotation*

	Without rotation		With rotation	
	mean value	standard deviation	mean value	standard deviation
Frequency	24 8	4 5	29 4	7 0
Total amplitude (mm)	201 0	54 9	233 6	50 6
Max eye speed of slow phase (deg/sec)	37 4	9 7	54 5	11 0



g 3 The recorded wave of cortical OKN without endular rotation (*upper*) and with pendular rotation (*lower*) Frequency total amplitude and eye speed of slow phase increased w th pendular rotation

f R OKN and L OKN form a mirror image ig 5 depicts the wave shown in Fig 4 Fol owing the example of R OKN the OKN was licited markedly when the slow phase of)KN and the compensatory eye movement in duced by the rotation from left to right are in the same direction On the other hand the OKN was inhibited when the two directions were mutually opposed After the test the sub jects mentioned that it was easy to look at the

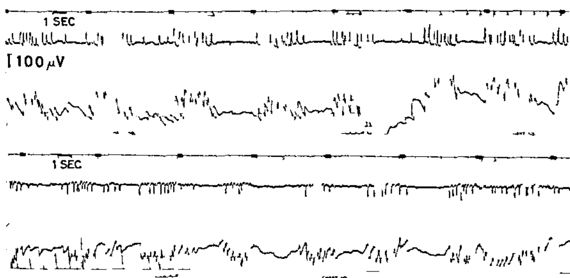


Fig 4 A recorded wave of R OKN (*upper*) and L-OKN *lower*) in a subject These subcortical OKN were alter nately promoted or inhib ted relating to a period of pendular rotation R-OKN and L-OKN form a mirror image Markers indicate the point where the subject was placed in the left hand position



Fig 2 The optokinetic cyl nder fixed on the rotatory chair

*s observed with eyes closed—or with the eyes open—in the dark

The subjects were tested sitting in the chair to which was fixed the optokinetic cylinder (Fig 2) in this way they were subjected simul taneously to pendular rotatory stimulation and to optokinetic stimulation The nystagmographic recording of horizontal eye move ments was done using time constants of 2 0 sec and 0 03 sec and a paper speed at 1 cm/sec or 10 cm/sec

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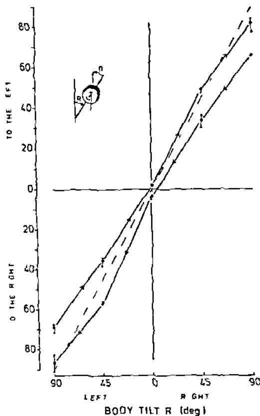


Fig 1 SV (angle β between adjusted luminous line and median plane of head) as a function of body position (R) which was changed by clockwise turning (*upper curve*) and counterclockwise turning (*lower curve*) The lines connect the means of the 6-min tests The vertical arrows indicate the mean readings of the first and last 2 min of each test The data refer to the average of 15 experiments 5 subjects were tested three times each The diagonal interrupted line indicates equality of SV and vertical

...ion has been reported by Clark & Graybiel (1964) for the postural vertical

The present paper offers a more detailed inspection of these phenomena of the SV

METHOD

The apparatus, a biaxial turning bed, and the general procedure were reported in a previous paper (Steinleitner, 1975) In the first series of tests the subjects were turned about a roll-axis (angle R) from an upright standing position (pitch angle pr_1) through 270° into a left or right side tilt of R 90° This was then the first tested position, for instance, R 90-left, which was followed by positions R 45 left, R 0, R 45-right and R 90-right

Subjects (Ss) were held for 6 min in each position while adjusting a luminous line to the SV 18 times 5 Ss (20–30 years of age) were tested, each three times

In a second series, 6 Ss (5 participants from series 1) were investigated in the tilt range beyond side tilt, being turned from R 0 direct to either R 110-right or R 160-right, thence to R 135 right During 4 or 6 min in each position they adjusted the line 12 or 18 times respectively Tests were carried out in two body positions pr_1, body upright, turning about dorsoventral axis of trunk, pr_3 trunk prone pitched with respect to head, turning about longitudinal axis of trunk The head was always turned in the same way, i e rolling about naso-occipital axis In control tests subjects were moved direct from R 0 to R 135 and checked there for either 8 and 12 min

RESULTS

SV at 5 positions between R 90-left and R 90 right altered clockwise or counterclockwise

Fig 1 and Table I demonstrate significant differences in the perception of SV at positions of the same R but attained in clockwise or counterclockwise sequence, i e reached from the one or the other direction of tilting (hysteresis of SV) At each position, SV readings

Table I *Statistical evaluation of significance of difference in SV at positions (R) attained in clockwise and counterclockwise process*

T test calculation is based on means of SV readings of first 2 minutes

R	*t*	df	*P*
90° left	6 786	4	<0 01
45° left	8 970	4	<0 01
0°	8 727	4	<0 01
45° right	22 090	4	<0 01
90° right	3 438	4	<0 05

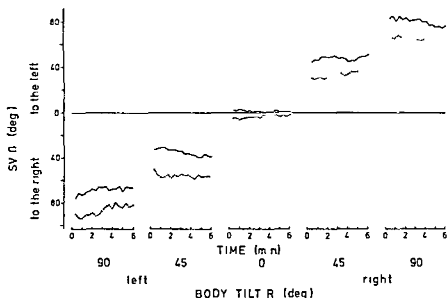


Fig 2 SV as a function of time and body position R Upper and lower curves refer to reaching of positions by clockwise and counterclockwise tilt respectively Same results as used for Fig 1 For further explanation cf Fig 1

deviated from a medium value in a direction opposite to the tilting direction (cp upper and lower curve of Fig 1)

The differences in SV vary in size with position R, being largest at the side positions (R 90) and smallest at the upright (R 0)

Decline of hysteresis in time The differences vary with time too (Fig 2) The distance between the two SV time curves of each position diminishes, due to different slopes of the curves But although they approach each other the curves do not reach the same level during

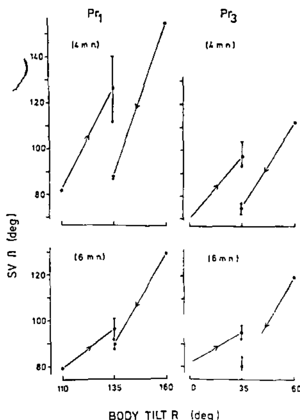


Fig 3 SV as a function of position (*R*) with body in upright or in trunk prone posture (pr_1 pr_3) R was changed to the final position R 135 from preceding positions R 110 and R 160 Vertical arrows indicate first and last time sections of the 4 and 6-minute experiments (cf Table III) *Left side* body posture upright pr_1 means of 4-min and 6-min experiments respectively *Right side* body posture trunk prone pr_3 mean of 4 min-and 6-min experiments respectively Average of measurements in 2 subjects (upper diagrams) or 4 subjects (lower diagrams) based on three repetitions of each experiments

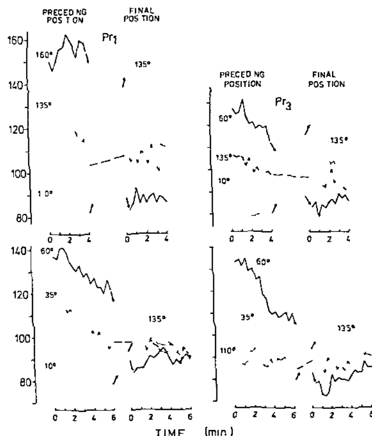


Fig 4 SV as a function of time and position (*R*) For further details cf caption to Fig 3 In addition the results of control experiments at R 135 are plotted (lasting 8 and 12 minutes respectively)

he 6 min of test time—not even in the upright osition where the intervening distance is mallest Thus also in this position there is n aftereffect lasting several minutes

V at positions R 135 right attained from ? 110-right and R 160 right

\gain there is a marked difference in SV (Figs 4, Table II) At final position R 135, reached rom preceding position R 110 the angle β of ,V increases steeply, reached from R 160 it drops below these values The curves of the ontrol tests (i e position R 135 tested for 8 nd 12 min respectively) are intermediate be-ween the two others This was expected for he preceding positions where the first 4 (resp 6) minutes of R 135 fit in between the R 110 nd R 160 data But it holds true also for the inal positions The SV curves of the final 4 (resp 6) minutes of the R 135 control are in between those succeeding the R 110 and R 160 curves Both these final curves converge and thus approach the control curve

Table II *Statistical evaluation of difference in SV at positions R 135 right attained from R 110-right and R 160-right*

T test calculation is based on the means of the readings of the first 80 sec of the 4 min experiments and of the first 120 sec of the 6-min experiments respectively

Exp time	*t*	df	*P*
pr1			
4 min	5 968	4	<0 01
6 min	8 265	7	<0 01
pr3			
4 min	4 333	5	<0 01
6 min	4 689	6	<0 01

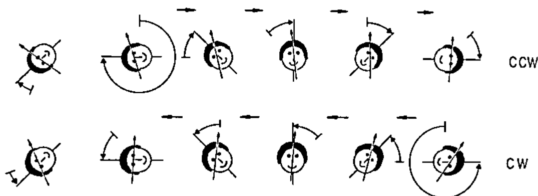


Fig 5 Illustration of SV perception at various positions (*R*) as changed in clockwise (*upper*) and counterclockwise (*lower*) sequence Straight arrows in front of head indicate SV as perceived in the first minute after reaching position Curved arrows refer to change from preceding position

Effect of prone position of trunk is obvious The curves obtained for body in pr_1 (upright at R 0) and pr_3 (trunk prone at R 0) differ from each other In pr_1 the difference in SV between R 110 and R 160 is more pronounced

DISCUSSION

The perception of SV is affected by the preceding tilt In a clockwise sequence of positions, for instance, deviation of the SV is unterclockwise (from a medium value at the ined position) In general terms, the SV viates in a direction converse to that of position process, which implies perception of the SV as if the body were in a more advanced tilt position of the process than it actually is (Fig 5) These results correspond to the findings of Clark & Graybiel (1964) on postural after-affects After spending 2 min (max) in tilt positions, the subjects were supposed to turn themselves back into the "upright" posture However, they failed, stopping before reaching the true upright, that is, the posture perceived as upright deviated from the true upright in the direction of the former tilt position, and opposite to the direction of movement Deviations were more pronounced in labyrinth defective subjects

A description of our results in terms of A and E phenomena is difficult Data in the upper curve of Fig 1, for instance demonstrate on the right a tendency towards an E effect but on the left an A tendency The opposit tendencies are present in the lower curve N such denotation can be applied to the devia tion in the upright position (R 0) This ex emplifies that these connotations are of littl explanatory value as far as the psych physiology is concerned

There is sound evidence linking these fin ings to the influence of the body's somator ceptor system The hysteresis difference (SV decreases with time, as a result of the dif ferent slopes of the two SV time courses in volved (Figs 2, 4) The time courses of SV have been interpreted as a function of adapta tion of the somatoreceptors, interacting wi the labyrinth position receptors (cf introduc tion) The process of adaptation may be af fected by the preceding tilt position Due to preadaptation of those parts of the somator ceptor system already stimulated in the pre ceding positions, the integrated informatio from all somatoreceptors, as fed into percep tion, may be biased by the newly involve somatoreceptors Thus the integrated infor mation indicates a more advanced state of th If, for instance, the subject is turned from a left tilt towards the normal symmetry pos tion, the right hand somatoreceptors dominat because of the adaptation of the left hand ones, so that the somatoreceptor system as a whole indicates an advance position not ye

ttained The subjects in our experiments e adjusting the SV as if they were in a more vanced position of the tilt process, in the periments of Clark & Graybiel subjects stop fore reaching the correct upright posture

The differences in SV (hysteresis) decline ntinuously for several minutes, due to ap oximation of the adaptational states of the matoreceptors The hypothesis that the matoreceptors are the main source of after-fects and hysteresis is supported by the find gs of Clark & Graybiel, that labyrinth efective subjects show a stronger aftereffect an normal ones

The more pronounced temporal change of V at R 135 indicates a stronger influence of omatoreceptors than below R 90 This cor esponds to the conception of a declining ef ectiveness of labyrinth position receptors eyond R 90 (Schone & Udo de Haes, 1968, 971)

Our interpretation of the hysteresis phe omenon is not intended to exclude the possi ility of contributions of other factors in gen rating these aftereffects

ACKNOWLEDGEMENT

Ve want to express our gratitude to Prof Dr I Kohler Psychol Inst Univ of Innsbruck) for his interest and upport of the work We wish to thank Mrs Phyllis Rechten for helping in the translation and Renate Alton or her co operative technical assistance

ZUSAMMENFASSUNG

Die subjektive Vertikale (SV) wurde in verschiedenen

...

Ahrlinks) oder im Gegen Uhrzeigersinnfolge (beginnend mit R 90-rechts) Die Stellung R 135 rechts wurde von R 110-rechts her eingestellt oder von R 160-rechts Die subjektive Vertikale hangt von der vorangehenden Stellung ab (Hysteresis) Bei Stellungswechsel im Uhr e pers nn be sp el ...

Hysteresisunterschiede in der Wahrnehmung der Stellung und der subjektiven Vertikalen durch adaptive Prozesse im Somatorezeptorensystem bedingt sind die mit dem Einfluß der labyrinthären Rezeptoren auf diese Vorgange interferieren

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SUCCESSFUL TRANSFER OF ADAPTATION ACQUIRED IN A SLOW ROTATION ROOM TO MOTION ENVIRONMENTS IN NAVY FLIGHT TRAINING

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Abstract Two flight students grounded for the reason they were highly susceptible to motion sickness completed their training after gradually adapting to 10 rpm achieved by executing head movements during small stepwise increases in angular velocity Subject 1 executed a total of about 77 000 head movements within a period of 5 months and Subject 2 executed about 108 000 head movements within a period of 42 days The transfer of adaptation acquired in the laboratory to most motion environments aloft was good the notable exception involved weightless maneuvers in the case of Subject 1 Both were on flight status when contacted recently The opportunity was taken to assess the current motion sickness suscepti ty in Subject 1 in the fall of 1975 He reached our (mild) on sickness endpoint in the rotating room at 17 rpm average endpoint is 7–8 rpm Some practical and -oretical implications are discussed

If a person riding in a slowly rotating room makes a head movement outside the plane of the platform s rotation, cross coupled accelerations are generated which stimulate the vestibular apparatus in an unusual way In the stationary state, head movements are associated with a pattern of accelerations that produce vestibular sensations consistent with the visual and proprioceptive sensations associated with these head movements past experience By adding rotation the bular sensations associated with the head movements now include those produced by the cross coupled This produces a situation where the vesti sensations are no longer consistent wit past visual and proprioceptive sensations subject s reactions to this unusual state grouped into two classes (Graybiel 1969) first class consists of reflexive reactions as nystagmus, tumbling or turning sensa and certain visual illusions The first seems to be a direct response to ve stimulation A second class of response directly related to the vestibular stimu constitutes the signs and symptoms of m sickness In as much as these signs and s toms have their immediate origins in vestibular systems, one must postulate a tative linkage between vestibular and vestibular systems as an important elem the causation of this form of motion sic (Graybiel, 1969) The signs and symp arising from this unusual vestibular sti tion have been well studied and a sen grading method is available (Graybiel e 1968)

It has been shown that subjects who form sufficient head movements at one crements can asymptomatically reach h

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lar velocities which would otherwise be in- erable (Graybiel & Knepton, 1972) By hav g the subject execute a schedule of head ovements at each increment in angular locity, one has a simple method of providing aptation to rotation This scheme is called incremental adaptation schedule If the ess level of the incremental adaptation hedule is excessively high, the incidence of otion sickness will force the termination of adaptation Although the relationship be een adaptation and motion sickness is not ll understood, it is possible, using suffici tly small increments in rpm to achieve adap- ion without overt symptoms of motion sick ss

Subsequent experience with incremental aptation (Graybiel et al , 1975) has shown at this acquired adaptation has two com nents The first to occur is a direction ecific adaptation which decays in hours after e cessation of rotation This direction specif- adaptation provides increased tolerance to tation only in the direction employed in the remental adaptation schedule It is also as ciated with a reduced tolerance to head ovements at zero velocity and an even lower lerance to head movements performed with tation in the opposite direction The rather pid decay of the direction specific adaptation veals a second component of adaptation hich is not direction specific and decays owly over days This second component of daptation is not associated with symptoms at ro velocity and does afford increased toler nce to head movements performed with rota on in the opposite direction This second

[illegible]

iators are dropped from flight training be- use of repeated episodes of severe air sick ss It is reasonable to assume that in their situation the stimulus level is so high that the prompt emergence of air sickness does not permit the occurrence of any significant adaptation A similar stimulation may be created in the laboratory by repeatedly exposing the subject to a high angular velocity without the benefit of incremental adaptation To test the practical usefulness of this phenomenon, it was decided to determine whether laboratory conducted incremental adaptation could be beneficial to student aviators with a history of severe air sickness

MATERIAL AND METHODS

Subjects for this experiment were two flight students who were dropped from flight training due to repeated episodes of severe air sickness Both students had a life long history of motion sickness Other than the unusual history of motion sickness, medical examination revealed two young, healthy adult males, both highly motivated to remain in the flight program By history and on the basis of their previous performance in the flight program, these two students demonstrated an incidence of air sickness far above average and one would expect a high susceptibility to motion sickness This suspicion was confirmed in both students where comprehensive vestibular testing revealed normal function with the exception of a very high susceptibility to motion sickness

The rotating device used in this experiment is the Slow Rotation Room I (SRR) at the Naval Aerospace Medical Research Laboratory in Pensacola Florida The experiment is conducted inside a windowless, air conditioned, circular room which is 10 feet in diameter and 7 feet high This room is attached to a large, high mass centrifuge that is capable of very smooth rotation at angular velocities from one to thirty revolutions per minute (Hixon, 1963)

By means of controlled vestibular stimulation each subject is well adapted to each increment of angular velocity The rotation is provided by the SRR rotating at constant angular

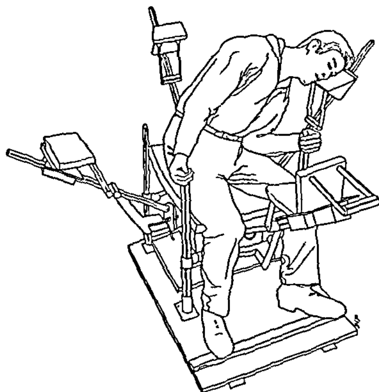


Fig 1

velocities The vestibular stimulation consists of paced head movements In this procedure the subject sits upright and upon command from a tape recorder he makes head move-
to the left, right, forward and back-
As shown in Fig 1, the angular dis-
of the head movement is controlled by the placement of pads in each direction of the head movement at an angle of 45 degrees from the vertical The subject moves his head in the desired direction until he lightly touches the appropriate pad The commands from the tape recorder specify a given direction every four seconds with the command to return to the upright following the initial command by 2 sec With this arrangement a discrete head movement is made every 2 sec and at the end of 480 such head movements (16 minutes) the subject is given a three to five minute rest period during which he sits quietly with his head in the upright position The incremental adaptation schedule will be designed on a daily basis by the authors with the objective of keeping the stress level as high as possible yet avoiding significant motion sickness Meas-

urements of the tumbling ('giant hand
lusion will be made at each new increme
angular velocity to estimate the intens
the vestibular stimulation The sever
motion sickness will be measured contir
using a previously described grading sy
(Graybiel et al , 1968), which is summari
Table I At the end of each daily rotation
movements will be immediately conduct
zero velocity to assess the level of acq
direction specific adaptation It has beer
posed that the acquired adaptation wh
not direction specific is continually overt
yet always lagging behind the acquisiti
direction specific adaptation (Graybiel e
1975) If a subject performs sufficient
movements at a given angular velocity f
then continue his head movements at
velocity without any incidence of motior
ness This occurs, presumably, because f
continued his adaptation to the rotatin
vironment for a period of time long enou
permit the decay of the more transient
tion specific adaptation to occur, even as
rotating If the stress level is properl

able I *Scoring severity of acute motion sickness*

itegory	16 points	8 points	4 points	2 points	1 point
iusea syndrome	Vomiting or retching	Nausea[a] II III	Nausea I	Epigastric discomfort	Epigastric awareness
in color		Pallor III	Pallor II	Pallor I	Flushing
old sweating		III	II	I	
creased salivation		III	II	I	
owsiness		III	II	I	
in					Headache
ntral nervous system					Dizziness Eyes closed ≥II Eyes open III

III=severe or marked II=moderate I=slight

sted, the subject will display minimal illu-ons at each new increment in angular veloc-/ and will transiently develop no more than ie or two motion sickness points throughout itation

At the conclusion of rotation the subjects iould remain essentially asymptomatic dur-ig the head movements at zero velocity If ie stress level is excessive the illusions will e more prominent, the motion sickness more -vere, and the post-run head movements will licit frank motion sickness Each subject be an at one rpm and worked his way to 10 rpm s quickly as possible

RESULTS

'he first attempt at adaptation was conducted ith Subject 1, whose motion sickness sus eptibility was somewhat lower than that of Subject 2 These results are displayed in Ta le II This first experiment was conducted ver a 7 day period and involved rotation on 6 ays Rotation was always in the counter lockwise (CCW) direction

On the first day Subject 1 reached 5 rpm and experienced no motion sickness through-out the day The subject executed a total of 7200 discrete head movements This cor responds to exactly 4 0 hours making head movements and was accomplished in 6 18 hours of rotation Illusions were not prominent at each new increment in angular velocity Due to a technical oversight, no post run head movements were performed on his first day

On the second day the subject reached 6 rpm and developed no more than 1 motion sickness point while rotating He performed a total of 5760 head movements (3 2 hours) in 6 85 hours of rotation Illusions were clearly present at first reaching 5 and 6 rpm After stopping the subject developed 5 motion sick-ness points in 115 head movements

On the third day the subject reached 8 rpm and displayed no more than 2 motion sickness points but remained at 1 point throughout most of the day He performed 6240 head move-ments (3 47 hours) in 7 30 hours of rotation Illusions were present but not prominent Dur-ing the postrun head movements the subject developed 6 motion sickness points in 90 head movements

On the fourth day the subject did not exceed 8 rpm He developed a maximum of 3 motion sickness points and displayed 2 points for much of the day He performed 4320 head movements (2 4 hours) in 7 0 hours of rota tion Illusions were present but not prominent at 6–8 rpm Upon stopping the subject de veloped 4 motion sickness points in 120 head movements

On the fifth day the subject reached 10 rpm He displayed a maximum of 2 motion sickness points at any time during rotation and was asymptomatic at 10 rpm The subject exe-cuted a total of 6720 head movements (3 73 hours) in 8 05 hours of rotation Illusions were detectable but not prominent Upon st(

Table II *Incremental adaptation, Subject 1*

		Angular Velocity (rpm)											Daily total	Average MS score	Post run HM	Po MS Sc
Date	Run day	0	1	2	3	4	5	6	7	8	9	10				
8 Jan 71	1 (CCW)		0 96[a]	0 96	1 92	1 92	1 44						7 20	0		
9	2						1 92	3 84					5 76	1	0 12	5
10	3						0 48	0 96	3 84	0 96			6 24	1	0 09	6
11	4							0 48	0 96	2 88			4 32	2	0 12	4
13	5							0 48	0 48	0 48	3 84	1 44	6 72	1	0 12	2
14	6										0 96	2 40	3 36	1	0 12	3
			0 96	*0 96*	*1 92*	*1 92*	*3.84*	*5 76*	*5 28*	*4.32*	*4.80*	*3.84*			*0.57*	
													Grand total 34 17			
6 May '71	1 (CCW)		0 96	0 96	1 92								3 84	0	0 24	1
7	2		0 12	0 12	0 12	2 88							3 24	1	0 24	0
10	3		0 12	0 12	0 12	0 48	1 92						2 76	0	0 24	0
11	4		0 12	0 12	0 12	0 12	0 48	1 80					2 76	1	0 24	1
12	5		0 12	0 12	0 12	0 12	0 12	0 48	1 92	1 92			4 92	2	0 24	2
13	6		0 12	0 12	0 12	0 12	0 12	0 12	0 12	1 92			2 76	0	0 24	3
14	7		0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 48	1 92		3 24	1	0 24	2
17	8		0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 96	3 84	5 76	0	0 24	0
			1 80	*1.80*	*2 76*	*3 96*	*2 88*	*2 64*	*2 28*	*4 44*	*2.88*	*3.84*			*1 92*	
													Grand total 31 20			
1 June '71	1 (CW)		0 96	0 96	0 96	0 96	0 96	0 96					5 76	1	0 24	2
2	2		0 12	0 12	0 12	0 24	0 24	0 48	0 96	0 96	0 96	0 96	5 16	1	0 24	1
			1 08	*1 08*	*1 08*	*1 20*	*1.20*	*1 44*	*0 96*	*0 96*	*0 96*	*0 96*			*0 48*	
													Grand total 11 40			

[a] 1 00=1000 individual head movements spaced at two second intervals

the subject developed only 2 motion sickness points in 120 head movements

On the sixth day the subject spent most of time at 10 rpm His displayed a maximum 2 motion sickness points and was asymp- by the end of the day The subject executed 3360 head movements (1 87 hours) in 4 57 hours of rotation Illusions were present but not prominent Upon cessation of rotation the subject developed 3 motion sickness points in 120 head movements

On the seventh and eighth days the subject was flown in an aircraft especially prepared for studying air sickness The subject displayed normal susceptibility which was interpreted as a significant improvement in his condition Of the various maneuvers employed, the subject was most sensitive to ' porpoising ' which involved a few seconds of weightlessness Several days later he participated in studies involving zero *g* parabolas of 30–45 sec duration Here he displayed such high air sickness suceptibility as to indicate that the incremental adaptation had afforded little p tection for this particular type of maneuver

Following the first incremental adaptati periodic measurements of motion sickn susceptibility were made to estimate the r of decay of the acquired adaptation All these tests were performed in the CCW dir tion, the same as that of the first increme adaptation At 12 days after the completion the first study, there was minimal waning the acquired adaptation At 33 days there significant waning and at 58 days the subj had returned to his previous baseline s ceptibility.

Dissatisfaction with the incidence of mot sickness in the first incremental adapta led to the decision to attempt a second sim experiment The objective was to examine t effects of lowering the stress level of the i cremental adaptation schedule so as to red the incidence of illusions and motion sickne while rotating This in turn would hopef reduce the incidence of motion sic

ible II *Incremental adaptation, Subject 2*

te	Run day	Angular Velocity (rpm) 0	1	2	3	4	5	6	7	8	9	10	Daily total	Average MS score	Post run HM	Post run MS score
Mar 71	1 (CCW)		1 92[a]	1 44	1 92	3 84							9 12	3	0 24	12
Apr 71	2		1 92	3 84	1 92								7 68	2	0 24	6
	3		1 92	2 40									4 32	1		
	4a		1 92	3 84									5 76	0	0 24	0
	5a			2 88	0 96								3 84	0	0 24	0
	6a			1 92	1 92								3 84	0	0 24	0
	7b			0 96	2 88								3 84	0	0 24	2
	8b				2 88	0 96							3 84	0	0 24	1
	9b				0 96	2 88							3 84	0	0 24	1
	10b					2 88	0 96						3 84	2	0 24	8
	11b					1 92	1 92						3 84	0	0 24	1
	12b					0 96	3 84						4 80	0	0 24	1
	13b						1 92	2 88					4 80	1	0 24	5
	14b							2 88	1 92				4 80	1	0 24	2
	15c							0 48	0 48				0 96	4		
	16b			0 48			0 48		2 88				3 84	2	0 24	7
	17b		0 12	0 12	0 12	0 12	0 12	0 12	1 68	2 88			5 28	2	0 24	8
	18b		0 12	0 12	0 12	0 12	0 12	0 24	0 48	1 20	0 96		3 48	2	0 24	7
May '71	19b		0 12	0 12	0 12	0 12	0 24	0 24	0 72	0 48	1 44		3 60	4	0 24	6
	20b		0 12	0 12	0 12	0 12	0 24	0 24	0 24	0 48	2 16		3 84	3	0 10	10
	21c		0 12	0 12	0 12	0 12	0 24	0 24	0 24	0 48	1 20		2 88	2	0 08	6
	22c		0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 36	0 96	2 28	2	0 21	6
	23b		0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 36	1 20	2 52	3	0 20	7
	24c			0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 24	2 40	3 48	2	0 15	9
	25		0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 12	0 24	0 72	1 92	1	0 18	7
			8 64	*18 84*	*14 52*	*14.52*	*10 56*	*7 80*	*9 12*	*6 00*	*6 96*	*5 28*			*5 00*	
													Grand total 107 24			

1 00−1 000 individual head movements spaced at two second intervals
10 mg d amphetamine sulfate, orally
5 mg d amphetamine sulfate, orally
2 5 mg d amphetamine sulfate orally

caused by the post run head movements In this design the daily head movements always started at 1 rpm This second CCW incremental adaptation was started 80 days after the completion of the first

On the first day Subject 1 reached 3 rpm and was essentially asymptomatic throughout the day He performed 3 840 head movements (2 13 hours) in 3 50 hours of rotation Illusions were not noted Upon the cessation of rotation, the subject developed only 1 motion sickness point in 240 head movements

On the second day the subject reached 4 rpm and briefly displayed 2 motion sickness … m He per- … 8 hours) in … were not detected Upon stopping the subject remained asymptomatic through 240 head movements

On the third day the subject reached 5 rpm and briefly displayed a single motion sickness point at 1 rpm He performed 2 760 head movements (1 53 hours) in 2 4 hours of rotation Illusions were not reported After stopping, the subject remained asymptomatic during 240 head movements

On the fourth day the subject reached 6 rpm He remained essentially asymptomatic but transiently developed 2 motion sickness points after a momentary power failure The subject executed 2 760 head movements (1 53 hours) in 2 4 hours of rotation Illusions were not reported Upon stopping, the subject developed a single motion sickness point in 240 head movements

On the fifth day the subject reached 8 rpm

He was intermittently symptomatic, displaying one or two points for much of the day The subject executed 4920 head movements (2 73 hours) in 3 95 hours of rotation Illusions were not noted Upon halting, the subject developed a single motion sickness point in 240 head movements

On the fifth day the subject reached 8 rpm He was intermittently symptomatic, displaying one or two points for much of the day The subject executed 4920 head movements (2 73 hours) in 3 95 hours of rotation Illusions were not noted Upon halting, the subject developed 2 motion sickness points in 240 head movements

On the sixth day the subject did not exceed 8 rpm and remained asymptomatic throughout rotation He performed 2760 head movements (1 53 hours) in 2 5 hours of rotation Illusions were not noted Upon stopping, the subject developed 3 motion sickness points in 240 head movements

On the seventh day the subject reached 9 rpm and intermittently scored 2 motion sickness points on two occasions during the day He performed 3240 head movements (1 8 ours) in 2 85 hours of rotation Illusions were t noted Upon the cessation of rotation the bject displayed 2 motion sickness points in -40 head movements

On the eighth day, the subject reached 10 rpm and he briefly displayed 2 motion sick ness points upon initially reaching 10 rpm He performed 5760 head movements (3 2 hours) in 4 3 hours of rotation Illusions were absent Upon stopping, the subject remained asymptomatic in 240 head movements

Although the second incremental adaptation employed slightly fewer head movements than the first, the succesful adaptation to 10 rpm was accomplished with less motion sickness and a much lower incidence of illusions Pro vocative tests to assess motion sickness sus ceptibility (Kennedy & Graybiel 1965) were conducted at 7 and 8 days after the comple tion of the second incremental adaptation When compared with the earlier baselines before both adaptation experiments there w substantial reduction in motion sickness s ceptibility When this test was conduct the direction opposite to that of both mental adaptations, i e clockwise there no evidence of transfer of adaptation to opposite direction This result was surpris since some transfer was expected To ex this possibility in greater detail, it was d to conduct a clockwise (CW) increme adaptation

Subject 1 started a CW incremental ada tion 14 days after the conclusion of the se CCW adaptation experiment The techn was to be the same as the second CCW e periment and the goal would be 10 rpm CW

On the first day the subject reached a s prising 6 rpm and displayed a maximum 2 motion sickness points during the day I performed 5760 head movements (3 2 h in 4 75 hours of rotation Illusions were ported upon first reaching 5 rpm Upon s ping, the subject developed 2 motion sick points in 240 head movements

On the second day the subject reached rpm At one point he briefly developed 3 tion sickness points but was back to 1 p within an hour He performed 5160 h movements (2 87 hours) in 4 45 hours of r tion Illusions were not present Upon s ping, the subject developed 1 motion sick point in 240 head movements The subje rapid progress to 10 rpm CW was most lik due to transferred adaptation from the sec CCW experiment

At this point the subject was returned flight training but due to a recurrence o chronic sinusitis, he did not immedia return to flying status Because of some ficulty in controlling this chronic sinus Subject 1 was temporarily suspended from ing However, the problem finally subsi and the subject finished flight training little difficulty He is presently in an op tional flying billet and periodic follow-up indicated no abnormal incidence of mo sickness In the fall of 1975 Subject 1 bri

ırned to Pensacola and ıt was possıble to ın measure hıs motıon sıckness susceptıbıl

At thıs tıme tıme he dısplayed at typıcal :d) endpoınt at 17 rpm whıch ıs well above average of 7–8 rpm

'he ıncremental adaptatıon of Subject 2 ısısts of a sıngle, lengthy adaptatıon to rpm CCW Some dıffıculty was antıcıpated :hat Subject 2 was found to be one of the st motıon sıckness susceptıble ındıvıduals :r tested at the Naval Aerospace Medıcal search Laboratory The plan was essentıal the same as employed wıth Subject 1 The ults of thıs experıment are dısplayed ın ble III

)n the first day Subject 2 reached 4 rpm ın !5 rpm ıncrements He was symptomatıc al ›st the entıre day, averagıng about 3 motıon :kness poınts and once reachıng 8 poınts : executed 9120 head movements (5 07 ›urs) ın 9 5 hours of rotatıon Illusıons were ways present and promınent above 3 25 rpm pon haltıng, the subject developed 12 motıon :kness poınts ın 240 head movements Al- ough the stress level was ıntentıonally de- ;ned to be low, ıt was stıll excessıve for thıs ghly susceptıble subject

On the second day the subject dıd not ex :ed 2 5 rpm The subject was symptomatıc uch of the tıme and averaged 2 motıon sıck :ss poınts He performed 7680 head move ıents (4 27 hours) ın 8 7 hours of rotatıon lusıons were promınent above 1 75 rpm 'pon haltıng, the subject developed 6 motıon :ckness poınts ın 240 head movements Agaın he stress level was excessıve

On the thırd day the subject dıd not exceed 75 rpm Throughout the day he dıd not de- 'elop more than 1 motıon sıckness poınt He ›erformed 4320 head movements (2 4 hours) n 3 25 hours of rotatıon Illusıons were less ›romınent than on the prevıous 2 days Due to echnıcal dıffıcultıes, post run head move nents were not conducted on thıs day In vıew ›f the unusually slow progress toward 10 rpm, t was decıded to attempt the ıncremental ıdaptatıon wıth the use of an effectıve antı motıon sıckness drug, d amphetamıne sulfate (Graybıel & Wood, 1969, Wood & Graybıel, 1972)

On the fourth day the subject dıd not exceed 2 rpm Thıs run employed 10 mg d ampheta mıne sulfate p o and the subject was asymp- tomatıc throughout the day He performed 5760 head movements (3 2 hours) ın 5 4 hours of rotatıon Illusıons were present but not promınent Upon stoppıng, the subject re- maıned asymptomatıc ın 240 head movements Usıng thıs technıque the subject gradually worked hıs way to 7 rpm, reachıng ıt on the sıxteenth day The subject generally averaged one or two motıon sıckness poınts, though the trend was toward greater motıon sıckness at hıgher angular velocıtıes He performed ap- proxımately 4000–5000 head movements per day Illusıons remaıned present and were oc- casıonally promınent Post-run head move ments were assocıated wıth gradually ıncreas- ıng motıon sıckness scores, reachıng 8 poınts on the sıxteenth day At thıs poınt the drug was combıned wıth the technıque of startıng all daıly head movements at 1 rpm

From the seventeenth through the twenty- fourth day the subject gradually worked hıs way to 10 rpm D amphetamıne sulfate and the technıque of startıng all daıly head movements at 1 rpm were contınued The subject was con tınually symptomatıc and averaged about 2 motıon sıckness poınts durıng each day He performed about 3000 head movements per day Illusıons were almost always present but rarely promınent

The post run head movements produced from 6–10 motıon sıckness poınts

On the twenty fifth day the subject agaın reached 10 rpm and no drug was employed for thıs run The subject dısplayed only 1–2 mo tıon sıckness poınts throughout the day He performed 1920 head movements (1 07 hours) ın 2 4 hours of rotatıon Illusıons were pres ent but not promınent at 10 rpm Upon stop pıng, the subject developed 7 motıon sıckness poınts ın 180 head movements

Subject 2 subsequently returned to flight

training which he completed with no usual difficulty with air sickness He is presently in an operational flying billet and periodic fol low up has not revealed any abnormal incidence of air sickness

DISCUSSION

On the basis of the results of this experimental probe and the reports of other investigators (Dowd & Cramer, 1967), it is altogether likely that the incremental adaptation to 10 rpm was beneficial to the two flight students Firm con clusions are difficult to achieve with such a limited number of subjects

However, both subjects have long felt that the adaptation experiments were of considerable aid in completing their flight training It is clear that established laboratory tests demon strate that these two subjects were able to reduce their motion sickness susceptibility while associated with the Laboratory The relationship between the reduced motion sickness susceptibility upon leaving the Labora tory and the subsequent success in flight train ing requires more careful examination If, for xample, the same tests that were used to easure motion sickness susceptibility before and after adaptation could be continued through flight training, then one might gain some insight into the relationship between incremental adaptation and improved flight training performance To obtain a better comparison, students with comparably high susceptibility might be paired, one receiving in cremental adaptation and the other continuing in the flight program It would also be useful to periodically measure the motion sickness susceptibility of normal students as they pro gress through training It is probable that the motion sickness susceptibility of student aviators as a group decreases as they progress through training This effect must be con sidered before estimating any improvement at tributed to vestibular adaptation

There are several aspects of the data which deserve additional comment In the case of Subject 1, there was good transfer of labo tory acquired adaptation to flight maneu with the exception of these involving weig lessness A possible explanation may lie in fact that weightlessness exerts a major ef upon the otolith apparatus whereas the ves ular stimuli employed in the laboratory p marily condition the semicircular canals S one would not except a conditioning proc involving the canals to necessarily transfer the otolith apparatus, it is then underst able that the incremental adaptation to 10 r afforded no protection against weightlessne After the second CCW incremental adap tion of Subject 1, a motion sickness susc tibility test failed to reveal any significa adaptation to the opposite direction This surprising in view of earlier work (Reason Graybiel, 1969) which predicted substan transfer When a subsequent CW increme adaptation was conducted, the rapidity of subject s progress clearly implied considera transfer from the previous adaptation in opposite direction The only explanation pr ently available is that this test was conduc prematurely, before the complete disappe ance of the direction specific component adaptation

In the case of Subject 2, the facilitation adaptation through the use of drugs represe an interesting possibility that requires furt investigation Whether d amphetamine s fate promotes adaptability by suppressing m tion sickness cannot presently be proven W Subject 2, the decision to employ a drug w largely based on the desire to continue the cremental adaptation The initial response the drug was significant but due to the s ject's complaints of nervousness the dos was gradually lowered What effect increa dosage would have had on the increased m tion sickness symptomatology above 6 rpm not known

The practical value of incremental adap tion is that it provides a method of reduc air sickness susceptibility which altho time consuming, can be accomplished safe

ıly, and inexpensively with a minimal in ment in equipment This method does not ılve the elicitation of motion sickness Al ıgh the data presented here were collected ı slowly rotating room, there is no reason ' the technique could not be arranged to ze a simple rotating chair

rom a theoretical viewpoint, incremental ptation represents a flexible experimental ınique for gaining insight into the progress vestibular adaptation By regulating the ection of rotation the angular velocity, and number of head movements, the investi or can reliably generate a variety of ves ılar stress levels that range from the sub- eshold to those which are frankly provoca- : of motion sickness The relationship be :en motion sickness and adaptation is not ll understood It has been reported that ıptation can occur without incurring signifi ıt motion sickness (Graybiel et al , 1968) l this has again been shown in these results ıwever very little is known about the cir ımstances promoting optimal adaptation om existing data it seems probable that mo- n sickness is not a necessary element of aptation This still allows the possibility ıt motion sickness actually retards adapta n which would seem to explain the be- viour of some student aviators If this ɔuld be true then the present method of ıking decisions on the presence of early or ld motion sickness symptomatology may 't prove to be very efficient In the present sign the presence or absence of the tum ng illusion was noted with the initial head ɔvements at each new increment of angular locity Although this information can be 'nerally related to the adaptation process it not presently clear how it alone might be 'ed to establish an incremental adaptation :hedule In summary, two well motivated ight students with a lifelong history of mo- n sickness were referred to the Laboratory 'e to persistent air sickness of such seventy ı to jeopardize their continued participation the flight program By executing numerous paced head movements on a rotating room of gradually increasing velocity, both students substantially reduced their motion sickness susceptibility to the laboratory rotating en vironment After developing an essentially asymptomatic tolerance to 10 rpm through the technique of incremental adaptation, they returned to the flight program Both students completed flight training and are presently in operational flying billets where they have ex perienced no unusual incidence of motion sickness In these two cases it was possible to employ a recently described adaptation to vestibular stimuli which permits the effective transfer of reduced motion sickness suscepti bility from the laboratory rotating environment to an operational flight situation

ZUSAMMENFASSUNG

Zwei Flugschuler, fluguntauglich wegen starker Anfällig keit fur Luftkrankheit vollendeten ihre Ausbildung nachdem sie sich allmahlich an 10 rpm adaptiert hatten Sie erreichten dies indem sie Kopfbewegungen bei schrittweise vergroßerten Winkelgeschwindigkeiten ausfuhrten Testperon 1 führte insgesamt etwa 77000 Kopfbewegungen aus in etwa fünf Monaten und Test person 2 etwa 108000 in 42 Tagen Die Ubertragung der im Labor erworbenen Adaptation auf die meisten Bewegungsformen im Flug war gut die bemerkenswerte Ausnahme betraf Manover mit Schwerelosigkeit im Falle der Testperson 1 Beide waren flugtauglich als sie kurz lich befragt wurden Die Gelegenheit wurde benutzt die gegenwartige Anfälligkeit für Luftkrankheit bei Test person 1 im Herbst 1975 festzustellen Er erreichte uns ren (schwachen) Luftkrankheitsendpunkt in der rotieren den Testkammer bei 17 rpm der mittlere Endpunkt liegt bei 7–8 rpm Einige praktische und theoretische Folgerungen sind diskutiert

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ULTRASONIC IRRADIATION OF THE GUINEA PIG LABYRINTH

Correlation of Morphology and Intralabyrinthine Temperature

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bstract The effects of high frequency ultrasound on the ner ear structures after irradiation of the lateral semi rcular canal have been analysed by means of light id electronmicroscopy Sudden as well as gradual iorphological changes in the vestibular sensory epithe um are described Damage to the connective tissue roma blood vessels and nerve fibres characterized the ironic phase Both cochlear morphology and hearing mained normal after irradiation of some behaviorally onditioned animals The temperature rise in the laby nth during continuous irradiation was studied The ex eriments indicated that the mechanical effects may be iore significant than the thermal in explaining the struc iral changes of the inner ear of guinea pigs The results re compared with previous extensive experience of ultra ound treatment of patients with Meniere s disease

Iigh frequency ultrasound can cause damage o biological tissues There is a considerable ariation between the susceptibility of dif erent tissues to the effects of ultrasound kin bone, connective tissue and blood essels are affected far less than nerve fibres nd sensory cells Of all human tissue, nerve issue is said to be the most sensitive to iltrasound (Fry & Fry, 1953, Åstrom et al, 961) This characteristic has been utilized in he treatment of Meniere's disease (Arslan, 955 James et al, 1960, Sjoberg et al, 1963, orensen, 1970, Dionne et al, 1972, Basek, 973) When aimed at the vestibular part of he inner ear reduction of the caloric response and improvement of the vertigo have also been achieved

Recently, we reported (Stahle, 1976) freedom from vertigo in 52% of the patients following one irradiation Complete cessation or a considerable decrease of vertigo was reported in 72% by Sorensen (1970) Basek (1973) using the round window technique according to Kossoff et al (1967) has reported success in 80% The main objection against the ultrasound method has been the tendency to postoperative hearing deterioration, which in spite of precise aiming of the beam has occurred in 35–40% of the cases (James, 1969, Stahle, 1976)

Several experimental studies on the effects of ultrasound on the inner ear have been performed in various species (James et al, 1964, Stahle, 1964, Dominok & Preibisch Effenberger, 1965, Giancarlo et al, 1965, Lundquist et al, 1971, Crysdale & Stahle, 1972, Stahle & Sugar, 1973) Degenerative changes have been found in sensory and supporting elements, secretory cells, blood vessels and nerve fibres Collapse of the utricle and obstructive fibrosis of the semicircular canals have been reported (Lundquist et al, 1971, Barnett et al, 1973), as well as alterations in Na^+ and K^+ concentrations in the endolymph (James et al, 1964) The acute morphological changes in the vestibular end

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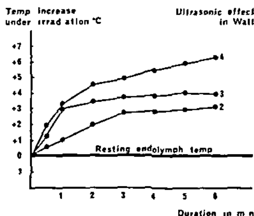


Fig 1 With micro-thermoelements the endolymphatic temperature was recorded continuously during the ultrasonic irradiation With an energy of 2 W a steady state temperature was reached 2 5°C above the resting temperature and with 3 W the temperature was elevated 3 5°C With 4 W however there was a continuous increase in the temperature and no steady state occurred within the *normal experimental range*

organs have been ascribed to mainly the complex mechanical effects of ultrasound (Lundquist et al , 1971)

The present study presents the morphological characteristics of the guinea pig labyrinth during the period 1–6 days, as well as 6–12 eks, post irradiation In order to get an of the thermal component of ultrasound, perature measurements have been made in he inner ear during irradiation Hearing tests before and after irradiation on behaviorally conditioned animals are included

METHODS

The 1 25 MHz Ultrapoint' apparatus (Johnson, 1967) was used The transducer was equipped with a cone shaped steel tip having an end diameter of 1 5 mm which was aimed at the bony wall of the lateral semicircular canal exposed through a postauricular approach No thinning of the otic capsule before irradiation was been done The effect was 3 watts and the irradiation time 3 min in all experiments

For comparison it must be mentioned that in a previous study we (Lundquist et al , 1971) used the Bristol Elliot 3 MHz machine This has an output effect of around 0 6 W The area of the tip of its probe is 0 03 cm² and the intensity 22 W/cm²

In the present study, where we have used the Ultrapoint machine, the probe s tip area was 0 018 cm² and the intensity about 17 W/cm² This means that a much higher effect has been used in this study than in the previous study, mentioned above Eight guinea pigs were used for histology (light microscopy and EM) One of them was sacrificed immediately after irradiation, the remaining between 2 hours and 6 days after irradiation Two behaviorally conditioned guinea pigs were irradiated in the same manner the survival times, however, set to 6 and 12 weeks *Three animals were used for intralabyrinthine* temperature recording In 2 animals the thermocouples (0 25 mm) were inserted in the lateral and anterior ampullae through minute openings in the otic capsule In one animal the thermocouples were inserted through the oval and round windows A 12 channel recorder was used to display the inputs from the thermocouples Several recordings were made simultaneously from each animal

RESULTS

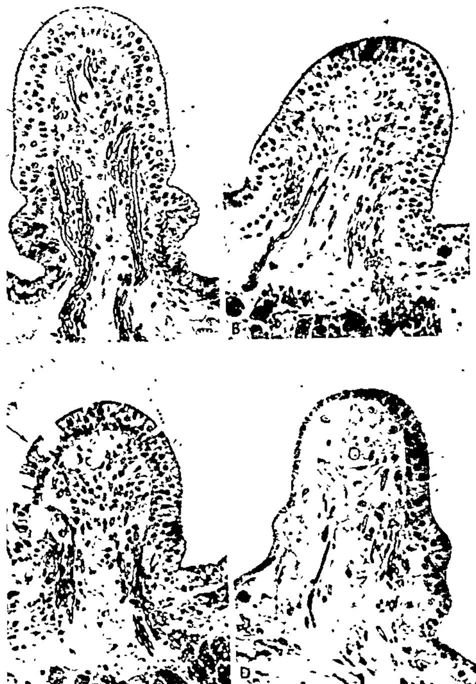
Temperature Measurements

In the animals used for intralabyrinthine temperature recording, several runs were obtained with energy levels ranging from 2–4 W (Fig 1) After 2–3 min of irradiation a plateau was reached at an increased temperature of +2 5°C over the resting endolymph temperature with 2 W, and +3 5°C with 3 W At an energy of 4 W, however, there was a continuous increase in the temperature, after 6 min becoming more than 6°C above resting temperature

Morphology

Acute changes

Light microscopy 0–2 hours after surgery the appearance of the lateral crista ampullaris was very similar to that of a normal specimen



g 2 Ultrasonic irradiation of the lateral semicircular nal ampulla with 3 W for 3 min (A) Control side with a rmal crista (B) 2 hours after surgery a slight formation vacuoles and a clearing of the connective tissue closest the basement membranes are the subtle signs of de- neration (C) Severely disrupted epithelium 2 days ter surgery The canal-side of the crista which has faced the ultrasonic transducer, shows the most severe damage (*arrow*) (D) 6 days after surgery the crista is healed, with a cuboidal epithelium covering the region devoid of sensory cells. The connective tissue appears irregular and a reduction of nerve fibres seems to have occurred

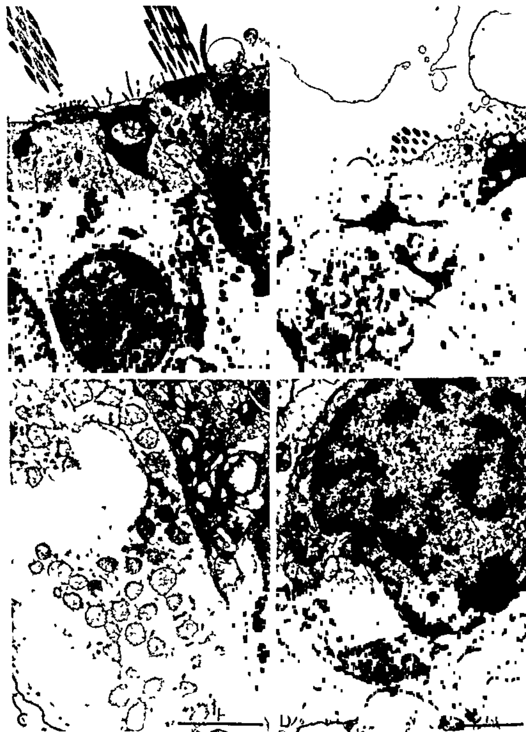


Fig 3 (A) Two hours after surgery Control side with a normal sensory cell type II as well as secretory cells (B) The signs of degeneration, 2 hours after surgery, are intracytoplasmic, with clearing between the mitochondrial cristae as well as formation of vacuoles in the apical cytoplasm (C) Also in the secretory cells (*left*) changes in the cytoplasm are present with clear zones and s irregularities of the granules The adjoining sensory (*right*) demonstrates a much more marked mitochon degeneration (D) Irregularities of the cytoplasmic m brane with infoldings of joining cells can be obse below the nucleus in this cell

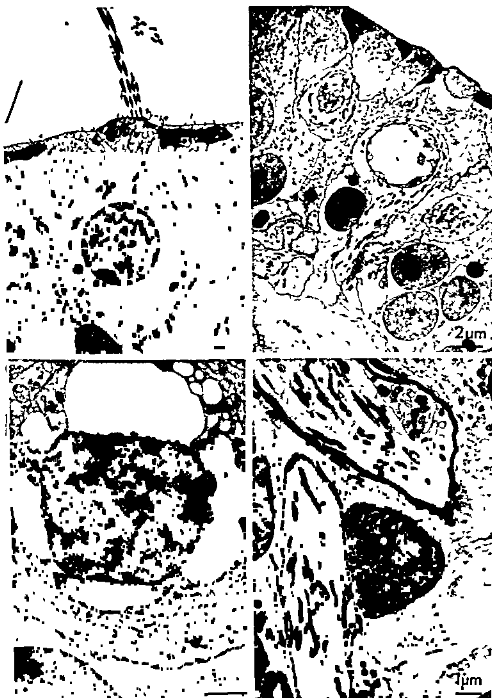


Fig 4 (A) One day after surgery Control side with normal sensory cell type I (B) Severely damaged epithelium with only few sensory cells remaining one of them appearing completely vacuolarized, but still partly remaining inside the epithelium lining and the surrounding nerve chalice (C) Some sensory cell nuclei are pyknotic and surrounded by an increasingly vacuolar cytoplasm (D) The nerve fibres appear normal

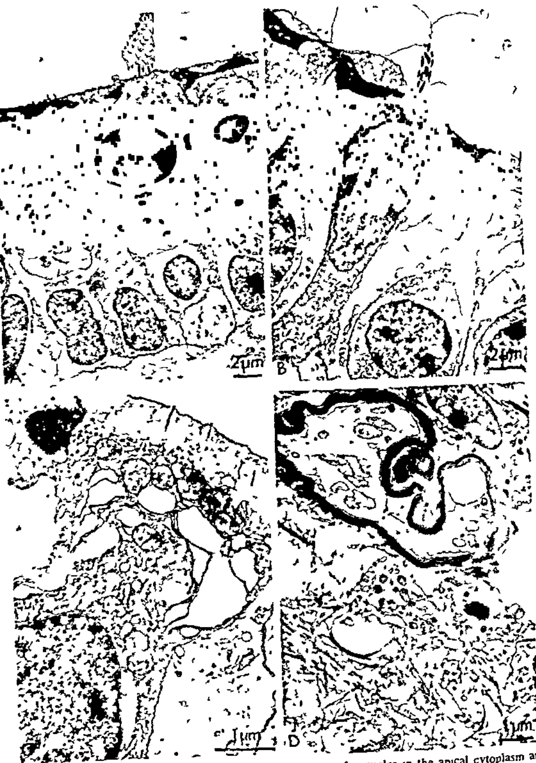


Fig 5 3–6 days after surgery (A) The utricles were nor

(C) Subtle changes could also be found where formation of vacuoles in the apical cytoplasm and almost normal ... were the only signs of degenera- ... fibres now ap- ... myelin sheath ... tive tissue ap- peared to be more fibrous than in the normal ampullae

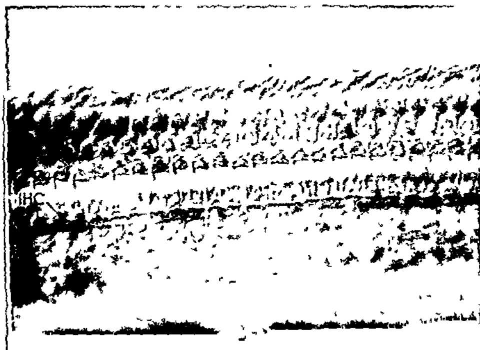


Fig 6 Surface preparation of basal turn cochlea 3 months after surgery, with completely normal sensory cell rows where the characteristic W shape of the sensory hair bundles can be recognized

There were, however, some signs of vacuolation of the epithelial lining but the sensory cells seemed basically intact The connective tissue stroma showed a slight edema The nerve fibres appeared normal (Fig 2B)

Electron microscopy In the sensory epithelium vacuoles were found in the cytoplasm and clear zones appeared in the mitochondria (Fig 3B) The secretory cells also were affected with formation of clear zones in the cytoplasm and granules (Fig 3C) The general cytoarchitecture remained normal, although in some cases a shrinking of the cell was observed and some irregular indentations of the cytoplasmic membrane were present The nuclei were morphologically normal—that is clumping of the chromatin or other marked changes in the nuclei and nuclear membranes were not observed (Fig 3D)

Subacute changes

Light microscopy 1–2 days after surgery the picture was markedly changed, characterized by a disrupted epithelium Many sensory cells as well as sensory hair bundles had disappeared completely These findings were more pronounced on the canal side of the crista, closest to the ultrasonic probe (Fig 2C)

Electron microscopy Distinctive from the very acute stage, marked changes now occurred in the cytoplasm and nuclei Many of the sensory cells were almost completely vacuolized (Fig 4B) or just remained as a cytoplasmic remnant surrounding a pyknotic irregular nucleus The secretory cells at this stage seemed to be less affected than the sensory cells themselves (Fig 4C) The nerves and nerve-endings were only slightly affected and there were normal appearing mitochondria in the axoplasm (Fig 4D)

Chronic changes

Light microscopy 3–6 days after surgery the irradiated cristae now seemed to undergo a healing process, with former denuded areas covered with a supporting epithelium devoid

of sensory cells On the utricular side the sensory cells remained, some of them appearing normal with sensory hair bundles still attached The connective tissue stroma appeared fibrotic and irregular and there seemed to be fewer nerves remaining

Electron microscopy On the utricular side of the epithelium where sensory cells still remained, there were signs indicating a continuing process of degeneration The sensory cells were often markedly shrunken, with a condensed cytoplasm and ruptured mitochondria (Fig 5B) The nerve chalices surrounding the type I cells seemed swollen with degenerating mitochondria The most subtle change found at this stage was the formation of vacuoles in the apical cytoplasm and of mitochondria (Fig 5C)

The connective tissue seemed much more fibrous than in earlier specimens Few blood vessels remained and the nerves now exhibited an irregular myelin sheath, vacuoles in the axoplasm and irregularities in the mitochondrial cristae (Fig 5D) The utricular epithelium in the whole series of animals irradiated with 3 W for 3 min was very little cted (Fig 5A) A detailed analysis remains made, however

ochlear changes

In the 3-watt series no cochlear changes were observed, either with behaviour audiometry or with morphology up to 3 months after surgery (Fig 6)

DISCUSSION

The biological mode of action of ultrasound is complicated In general it seems to be based on three concurrent factors—thermal, mechanical, and chemical

The thermal effect of ultrasound depends on an absorption of energy in tissue The amount of transmitted sound energy which is converted to heat is determined by the absorption coefficient of the tissue Bone has a high coefficient and therefore becomes hot (Bender et al, 1954, Baldes et al, 1958) A 10°C temperature rise in the human otic capsule during ultrasonic irradiation with a strength of 2.8 W has been recorded (Drettner et al, 1967) The substantial heating in the otic capsule in the target area in addition to the structural heating in tissues of varying acoustical impedance inside the labyrinth will raise the intralabyrinthine temperature In patients undergoing labyrinthectomy, a temperature rise to 40°C was recorded in the vestibule after irradiation with a strength of 2 W during 3 min In the lateral semicircular canal close to the irradiation spot the temperature rose to 44°C after 3 min irradiation with a strength of 2.5 W (Drettner et al, 1967)

The temperature rise of 2–3°C within 3 min in our experiments on guinea pigs can hardly explain the acute degenerative processes in the crista epithelium The critical temperature of the facial nerve in man is said to be about 46°C (James et al, 1964) The resistance to heating in neural tissue is further exemplified by the studies of Andersson et al (19 which showed that the sciatic nerve action potential in frogs was reduced by 50% first when the temperature reached the 48-51° range Against that background it seems more plausible to us in the present study to attribute the degenerative changes after ultrasonic irradiation mainly to *mechanical* factors Such an assumption is supported by the results of Fry & Fry (1953) showing that in experiments on hypothermic frogs the ultrasound produced its effects on nerve cells when the temperature in the cord was under 20°C They consider that the physical factor which should be considered fundamental in the mechanism is the viscous force acting between the submicroscopic structural components of the protoplasm and the surrounding fluid

The various effects of ultrasound upon the inner ear in Meniere surgery have been discussed in an earlier publication (Sjoberg et al, 1963) At that time we were inclined to attribute the biological effects mainly to the temperature rise From later studies we obtained

etailed information on the temperature in 's parts of the inner ear, which resulted a change to shorter irradiation periods Drettner et al, 1967) By reducing the irradia- time, the thermal influence upon the inner declined in importance As a result, the mechanical forces such as "agitation, cavita on and streaming" have become of more im- iediate interest as factors causing biological amage to the inner ear (Lundquist et al, 971)

Simple agitation may cause rupture of iaterials Sonic streaming, occurring even at ery low intensities, is thought to be one of the nost important modes of action in biological iaterial (James, 1963), it ruptures cell mem ranes—as in blood haemolysis—and breaks own bacterial membranes At lower inten- ities, important effects on the Na^+ and K^+ pump' in living cells have been observed nd are thought to be involved in improving he mechanism after irradiation for Meniere's isease (Hughes & Chow, 1964)

Cavitation, which takes place at high inten ities, means the production of microscopic ubbles of gas or vapour in liquids This henomenon occurs because, under certain onditions, such a low pressure may occur n the wave troughs that fluid particles are roken up and a hollow space containing a gas hase is formed From a morphological point f view it seems to be impossible to clearly eparate the cellular effects formed by the lifferent mechanical factors We have there ore grouped them under the heading ' mecha- ical effects" and our morphological findings vill be analysed against that background

At high energy levels with the ultrasonic robe in direct contact with the endolympha c space, it is possible to disrupt the cristae ompletely due to cavitation and streaming Lundquist, 1971) In the present investigation where the otic capsule was preserved, no such igns were observed, but almost immediately fter surgery one could with the electron microscope detect changes inside the mito- hondria and also the formation of small vacuoles in the cytoplasm These changes most likely seem to be due to the sonic stream ing, acting at the transition between the cyto- plasm and the mitochondrial membranes When, however, looking at the changes pres- ent at the subacute stage (1–2 days after sur- gery), it is obvious that the damaged mito- chondria are gone The large vacuoles formed in the cytoplasm of the degenerating and dis- appearing cells, express the severely altered metabolism of these cells The changes oc- curring in the nerve fibres are most likely due to the interstitial changes in the connective tissue such as fibrosis and diminished blood supply Even in the chronic stage (3–6 days after surgery), several severe signs of patho- logy are present in the sensory cells Some of the cells are very condensed in appearance and almost all their mitochondria are vacuo- lar In other cells the dominant findings are vacuoles formed in the cytoplasm as de- scribed in the immediate postoperative state This varying degree of degeneration occurring in the same sensory cell region is most likely due to an altered cellular metabolism as a re sult of the mechanical forces of ultrasound It seems clear from our present results that the ultrasonic irradiation not only gives rise to acute changes in the sensory epithelia but also to progressive changes commencing for several weeks after surgery

Our findings on experimental animals con tribute to the explanation of the favourable effects—as well as the failures—on Meniere patients Three events can be assumed to take place in the inner ear of man after irradiation (1) degeneration of the neuro-epithelium and its supporting structures mainly in the non- acoustic part, (2) degeneration of the secre- tory epithelium at the base of the cristae and (3) the creation of a small fistula in the wall of the membranous labyrinth All the three, separately, will be able to reduce the symp- toms caused by the endolymphatic hydrops A combined effect, however, seems to be more likely

(1) The reduction of the caloric reaction

after ultrasonic irradiation recorded in 70% of the Meniere patients (Stahle, 1976) can be explained by general degeneration of the cristae ampullares The reduction of the caloric response contributes to the diminishing of the vertigo Our experiments on guinea pigs have verified the existence of partial degeneration In 2 animals an almost complete degeneration of hair cells on the irradiated side of the crista was found after 6 days, while on the non irradiated side the cell population remained with minor changes visible in light microscopy Such a selective elimination of sensory cells on the cristae may explain why many patients have been improved though not completely free from vertiginous attacks

(2) A certain amount of endolymph and mucopolysaccharides is thought to be secreted from the cells in the transitional zone round the base of the cristae The degeneration of this epithelium following irradiation thus may contribute to the prevention of a labyrinthine hydrops Degeneration of the transitional cells, with formation of vacuoles and watery appearing cytoplasm, were reported in a previous experimental study on the immediate effects of ultrasound (Lundquist et al , 1971) changes in this area have been con -d in the actual investigation The import -ce of such a partial elimination of secretory epithelium in the prevention of a general labyrinthine hydrops remains obscure

(3) Morphological evidence of the creation of a permanent fistula through the membranous labyrinthine wall at the site of the irradiation has been presented by Sørensen (1976) Similar effect has been attributed to cryo surgery (Wolfson 1976) We have not analysed our specimens from that particular point of view but concentrated our interest upon the end organ morphology

The postoperative hearing loss found in around 40% of our patients with follow up time from one to ten years is thought to be the great shortcoming of the ultrasound method This deterioration is attributable partly to cochlear damage due to ultrasound but is also an expression of the natural course the disease (Stahle, 1976) In experimental mals the extensive ampullar lesions after sonic irradiation have not been by cochlear lesions Two behaviorally ditioned animals have presented normal ing and normal cochlear light microscop and 12 weeks postoperatively in spite of generation in the non acoustic labyrinth crude methods of evaluating changes in hearing acuity of research animals have t taken into account Possibilities remain h ever, that a real difference in sensitivit ultrasound exists between the normal amp in guinea pigs and the diseased end orga Meniere patients

ZUSAMMENFASSUNG

Die Auswirkungen auf die Innerohrstrukturen nach frequenter Ultraschallbestrahlung des lateralen F ganges wurden mit Licht und Elektronenm kro analysiert Sowohl akute als auch progressive mo logische Veranderungen im sensorischen epithel werden beschrieben Die Schädigung des gewebsstromas der Blutgefaße und Nervenfasern charakteristisch fur die chronische Phase Die Coc morphologie und das Gehor verblieben unbee normal nach Bestrahlung einiger gehortrainierter suchstiere Der Temperaturanstieg im Labyrinth wahrend der Bestrahlung fortlaufend beobachtet Die suche deuten darauf hin daß mechanische Effek die Klarung der Strukturveranderungen im Inne von Meerschweinschen bedeutungsvoller sind als male Die Ergebnisse wurden mit fruheren umfangre Erfahrungen mit Ultraschallbehandlung von Patiente Menierescher Krankheit verglichen

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THE EFFECTS OF MERCURIAL POISONING ON THE VESTIBULAR SYSTE

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Abstract The sensory epithelium with adjacent nerve endings and the secretory epithelium may both become damaged following mercury chloride intoxication Peripheral myelinated nerve fibres in the crista ampullaris showed signs of degeneration following chronic poisoning Ultrastructural alterations of the vestibular hair cells initially occurred in animals free from clinical signs of intoxication The sensory epithelium and occasionally the secretory cells were affected before signs of ultrastructural damage could be detected in the myelinated nerves

Neuro-otological disturbances following the se of drugs containing mercury have been own since antiquity Kussmaul (1861) ported vestibular dysfunction as one of the main symptoms following mercury poisoning Disease caused by mercury compounds used to be counted as a clinical rarity until several recent outbreaks of mass poisoning aroused public concern over its dangers (Goldwater, 1972, Takeuchi 1972)

Although many reports concerning mercury poisoning have appeared recently there have been only a few reports of neuro-otological observations on the intoxication caused by mercurials (Fryisaki et al 1971 Mizukoski et al , 1975, v Strupler, 1952) The increa in environmental pollution has further focus interest on the possible toxic effects of me cury

The purpose of the present study was t investigate whether mercury can cause vestib ular dysfunction by damage to the sensory o the secretory epithelia of the cristae ampul lares

MATERIALS AND METHODS

Sixty healthy young guinea pigs (250–350 g were used in this study The animals we treated with daily subcutaneous injections of mercury chloride ($HgCl_2$ as a 0 5% solut in sterile water) in doses ranging from 2 5 to 25 mg/kg b w (chronic intoxication 2.5–5 mg/kg b w , acute intoxication 7 5–25 mg/k b w) The total dose varied between 10 and 90 mg/kg b w

The survival time after the last injection varied from 6 hours to 1 month The control group of animals consisted of 9 healthy un treated guinea pigs

The clinical investigation of the animals and the procedure for fixation and morphological investigation have been described earlier by Anniko & Sarkady (1977) concerning cochlear pathology following exposure to mercury chloride

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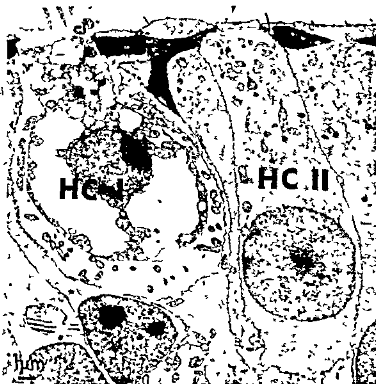


Fig 1 EM Chronic poisoning Vesicular degeneration of hair cell type I (HC I) while the hair cell type II (HC II) still is rather unaffected by the toxic effect(s) of mercury chloride

RESULTS

Sensory epithelium

In the investigated specimens of both types of poisoning, damage to hair cells type I and type II occurred However, acute intoxication affected the hair cells to a much lesser extent than chronic intoxication, in which case hair cells were frequently found in various stages of cellular disintegration The individual susceptibility of the hair cells to mercury chloride poisoning was, however, initially great because undamaged hair cells often appeared adjacent to severely degenerating hair cells of both types (Figs 1 and 2)

A complete loss of hair cells in the crista ampullaris or the macula utriculi did not occur Hair cells of type I were morphologically often affected before those of type II (Fig 1) In hair cells of type I the nuclear membrane sometimes appeared as contiguous with a membrane system entering the cellular cytoplasm and electron-dense material was found within this membrane system giving the appearance of a deposit which had been ejected from the nucleus Finally, both types of damaged hair cells showed vesicular degeneration of the cytoplasm and the cellular organelles The cytoplasm in some sensory cells, mostly type I hair cells, showed increased electron density A large number of vesicles of varying size appeared (Fig 3 A, B), as a result of which the cytoplasm became fragmented The nuclear membrane appeared wavy but was not discontinuous

The mitochondria were affected at an early stage of cellular damage and in many specimens the mitochondrial changes appeared as the first signs of hair cell pathology The mitochondria in the hair cells and the nerve endings showed similar structural changes In some mitochondria the two lamellae of a crista normally in close apposition appeared initially to be separate from each other, thus forming a minimal vacuole in the matricial space The cristae later fragmented without the formation

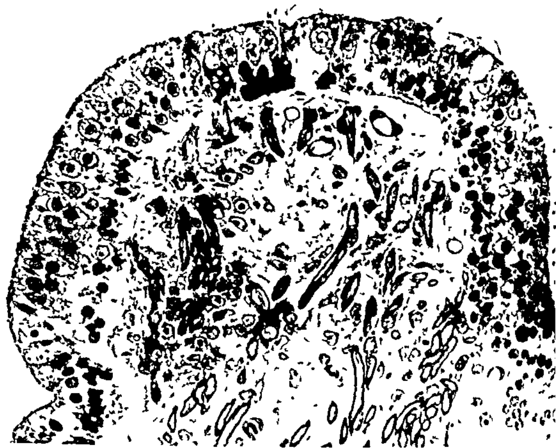


Fig 2 Light microscopy (LM) Crista ampullaris Degenerating hair cells of both types A dark osmiophil inclusion (*arrow*) is found below a hair cell (degene nerve ending?) ×120

myelin figures and large, confluent, non ainable regions of the mitochondrial matrix occurred, giving an empty appearance The mitochondrial outer membrane often showed herniations or even ruptures, a phenomenon not observed in the control material which underwent the same morphological preparation procedure

Following chronic intoxication, afferent and efferent nerve endings revealed signs of damage when the adjacent hair cell sustained ultrastructural damage (Fig 3C D) It proved impossible, however to establish which was damaged first, the sensory cell or the nerve endings Some of the nerve chalyces of type I hair cells became opaque as when a deposit of mercury compound is found in the neuroplasm In other, less damaged afferent nerve terminals, the mitochondria appeared swollen with destruction of cristae Similar morp logical changes also occurred in efferent ne endings, while the amount of transmi vesicles was estimated to be normal for a l time Nerve terminals adjacent to type II s sory cells were affected at the same time as innervating type I hair cells and the cytolog changes were similar Myelinated nerve fib in the connective tissue of the cristae amp lares were often damaged following chro poisoning (Fig 4) but the degree of the in vidual vulnerability varied greatly Ultrastr turally normal myelinated nerves could found in the same specimens as myelinat nerve fibres in different stages of dissolutio In the degenerating nerves the organelles the axoplasm were reduced in number or ev lost completely, while the myelin sheaths d integrated (Fig 4 A, B) Axons often contair

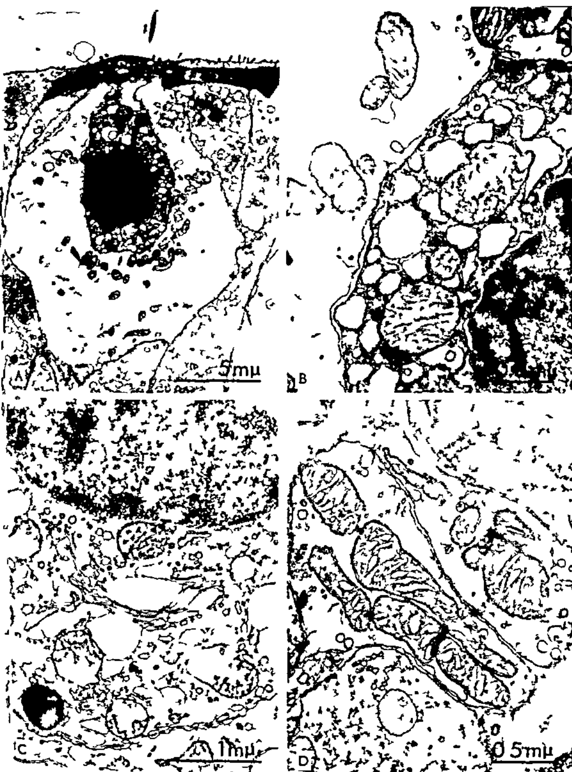


Fig 3 Electron microscopy (EM) Crista ampullaris *A* Chron c in ox ca on Type I hair cell show ng an electron dense cytoplasm containing vacuoles of vary ng s ze The ad acent afferent nerve chalyx s w dened but st ll con tains many mitochondria *B* Detail from F g 1A *C* Acute ntox cation The cytoplasm of a type I hair cell is ves cula ed and the mitochondria have lo t the r cristae forma on *D* Acute ntox ca on Afferent nerve ending The m ochondrial cristae appear separated from each other



Fig 4 EM Chronic intoxication Myelinated nerve fibres The axons contain foci with floccular dense bodies and granular debris

with floccular dense bodies and granular s

In acute intoxication both types of sensory cells were affected only very slightly while adjacent nerve terminals could appear ultra structurally changed However the individual susceptibility of the nerve endings to mercury chloride poisoning varied thus both normal and damaged nerve endings were observed in the same specimen Some specimens even appeared completely normal The variation in the nerve ending damage to individual nerve terminals within one specimen was relatively large thus enabling us to study the course of the destruction of the nerve ending under observation

When afferent and efferent nerve endings were damaged no difference in sensitivity could be observed between the two types The mitochondria were changed in the same way as described concerning the hair c Acute intoxication did not affect myelin nerve fibres

Secretory epithelium

Chronic mercury intoxication can somet cause alterations in the ultrastructure in dark cells of the secretory epithelium administration of small amounts of mercu each injection (2 5 mg/kg b w) did not r in any changes other than an increased culation of the dark cells normally conta secretory granules

Treatment with 5 0 mg/kg b w on occasion caused after 5–16 injection widening of the space between the long i digitations of the dark cell membrane w contain elongated mitochondria This wi ing could become so extensive that the cells appeared as almost separate from



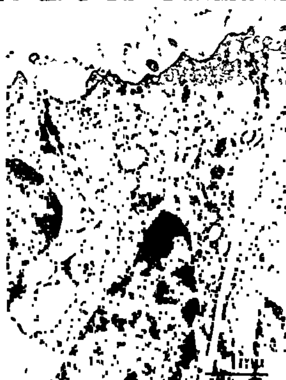


Fig 5 EM Chronic intoxication Dark cells of the secretory region of the crista ampullaris The cellular extensions are separated and the dark cells normally adjacent to each other show large intercellular regions except at the surface area towards the endolymphatic space

Fig 6 EM Chronic intoxication Oedema in cells of the secretory epithelium with a lighter cytoplasm than surrounding dark cells

Fig 7 EM Chronic intoxication Detail from the apical part of a dark cell showing an increase in electron density

other except at the margin towards the endolymphatic space (Fig 5) and the dark cell cytoplasm increased in electron density (Fig 7) Severely degenerating dark cells were not observed and ejection of damaged secretory cells into the endolymphatic space did not occur However, vacuoles of considerable size frequently occurred

Sometimes cystic formations were found below the secretory area of the dark cells Infrequently, cells with a lighter cytoplasm than surrounding dark cells were interspersed in the secretory region, in which case their cytoplasm appeared severely oedematous and lipoid like inclusion bodies could be observed (Fig 6)

Acute intoxication affected the dark cells of the secretory region only in rare cases, but when this occurred the morphological changes were similar to those found following chronic intoxication

The supporting cells or the secretory cells between the hair cells in the sensory epithelium were structurally unchanged There were likewise no changes in the transitional cells between the sensory area and the dark cells

DISCUSSION

Clinical observation of neuro otological disturbances following chronic poisoning by organic mercurials has revealed frequent signs of dysequilibrium as one of the main findings Mizukoski et al (1975) reported from their studies on 144 patients with Minamata disease following the Aganogawa River mass poisoning (organic mercurials) that signs and symptoms of disturbance of the vestibular system occurred in 90% of the investigated cases thus being a frequent symptom in mercury poisoning Similar observations were made by Kurland et al (1960) concerning the Minamata Bay poisoning in the 1950 s

The present ultrastructural study of the vestibular system showed that d image to both types of hair cells may occur but there was a great variation in individual susceptibility to the toxic effect(s) of mercury chloride Th secretory epithelium was seldom affected an the supporting tissue remained morpholog cally normal When damage occurred ne terminals degenerated at the same time as th adjacent hair cells

The serious symptoms of toxicity aris from chronic occupational exposure to i organic mercury and exposure to alkyl m cury compounds result from damage to th nervous system (McGregor & Clarkson 197 It is generally assumed that the patholog effects produced by mercurials result fro alterations in SH containing macromolecul within or on the surface of the target ce (Hughes, 1957, Passow et al, 1961, Rothste 1973)

The distribution of mercury within cells h been reviewed by Hopwood (1973) w reported that mercury binds very rapidly the cell membranes, so that the reaction completed within 2–3 minutes, suggesting t formation of typical covalent bonds A lo occurred of potassium ions from within t cells and sodium ion transport was inhibite The cellular uptake of glucose and glyce ceased Once the mercuric chloride is wi in the cell it will be distributed between t various components of the cytoplasm a the nucleus Norseth (1968) reported that t nuclei and lysosomes take up a considerab portion of added mercury Verity & Re (1967) found that both organic and inorgan mercuric compounds destroyed the lysosom membrane, with a subsequent release bound lysosomal enzymes into the cytoplas However, Verity & Brown (1970) report that mercuric chloride inhibited the activ of acid hydrolases released from the lys somes Findings of metallic mercury with the lysosomes of macrophages have be reported by Burge & Winkelmann (1970)

In the present investigation, degeneration sensory epithelium has been found in mo specimens in which the dark cells of the secr tory region engaged in the active and passi transport of ions for the preservation of

ormal composition of the endolymph appeared normal This indicates a direct action mercury chloride on the sensory cells Mitochondria were affected early both in the nerve ndings and in the adjacent hair cells Hopood (1973) reported that the overall effect of mercurials was to inhibit the respiration of the ll thus primarily affecting the mitochondria rcos et al (1967) showed that mercurial compounds give rise to mitochondrial swelling nerly et al (1967) found that the swelling f the mitochondria caused by mercurials was ue to a passive permeability to chloride ions They also found that mitochondria bound mercurials in proportion to the hydroxyl ion concentration (Jacobus & Brierly 1969)

Damage to nerve endings and myelinated nerve fibres was a prominent feature of the present investigation The difference between hronic and acute intoxication can probably be explained by the difference in the concentration and the duration of the exposure Relatively large amounts of mercury introduced suddenly may affect the excitable membrane directly whereas the sustained presence of smaller concentrations may lead to an accumulation of the poison within the cells and to a consequent disorder of metabolism

Ann ko & Sarkady (1977) interpreted some of their findings in myelinated cochlear nerves following chronic mercury chloride intoxication as a sign of demyelination According to Herman et al (1973) demyelination is not a morphological feature in mercury poisoning However Chang & Hartmann (1972) interpreted some of their findings as indicative of a demyelinating process Axonal degeneration with a loss of organelles in myelinated nerves has been reported in a number of conditions many apparently related to a suppression of oxidative energy metabolism including cyanide intoxication (Hirano et al 1967 Hirner 1969) and hypoglycemia (Webster & Ames 1965) Suppression of oxidative phosphorylation has been reported following mercury and other heavy metal administration causing swelling of the mitochondria (Southard et al 1974) While an axon is degenerating it becomes filled with floccular dense bodies and granular debris (Hirano 1972) Dense bodies consisting of lipid accumulations have been reported also in a number of lipoidases (Gonatas et al 1968 Guazzi & Van Bogeart 1969)

Pathological findings in the vestibular examination of patients with clinical signs of mercury intoxication have revealed a high frequency of gaze nystagmus (17%) and direction changing positional nystagmus (65%) (Mizukoski et al 1975) It was suggested that these nystagmus findings may be associated with diffuse lesions of the brain stem and the cerebellum Irregular nystagmus was observed also by Hunter & Russel (1954) though they did not discuss the underlying pathology

The present experimental study reveals that the vestibular hair cells of both types with adjacent nerve endings may become damaged following both acute and chronic intoxication Damage to the sensory epithelium occurred in the early stages without clinical signs of vestibular dysfunction The hair cells with adjacent nerve endings when damaged appeared so before the myelinated nerves became affected indicating a primary effect in the crista ampullaris

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ZUSAMMENFASSUNG

Das sensorische Ep thel um mit den angrenzenden Nervenenden sowie das sekretorische Ep thel um können bei zufolge von Mercurium-Chloridberauschung bescha d gt werden Peripherische bemarkte Nervenfasern in der Crista ampullaris w esen auch Ze chen von Degenerat on auf Ultrastrukturelle Veranderungen in der Crista ampullaris konnten in T eren die ke n Ze chen von Berauschung aufw esen vorkommen Das sensorische Ep thel um wurde beschad gt bevor Zeichen von ultrastrukturellen Veranderungen in den bemarkten Nerven entdeckt werden konnten

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BILATERAL NASAL VASCULAR RESPONSES TO UNILATERAL SYMPATHETIC STIMULATION

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bstract Unilateral preganglionic cervical sympathetic imulation in the anaesthetized cat evoked vasoconstric on in both nasal cavities dependent on stimulation equency Vasoconstriction in the contralateral cavity as 15–20% of that on the stimulated side Similar find were obtained on unilateral Vidian nerve stimula on Vasoconstriction evoked in the sympathectomized asal cavity by stimulating the opposite cervical chain was duced but not abolished by sectioning the posterior asal and ethmoidal nerves of the stimulated side It is ggested that vasoconstrictor fibres reach the opposite avity either in these nerves or by way of blood vessels ut more likely that sympathetic fibres from these path ays innervate blood vessels which supply both nasal avities

Bilateral hyperaemia and decreased nasal patency have been reported in man following unilateral cervical sympathectomy and stellate ganglion block (Bertein 1929, Stocksted & Thomsen 1953) In contrast to Undritz (1929) and Anggård & Densert (1974), Franke (1966) and Malm (1973) reported bilateral vasocon striction and increased patency in the nose of the dog and cat on unilateral cervical sympa thetic stimulation The contralateral responses were between 6 and 12% of the responses in the ipsilateral cavity Richerson & Seebohm 1968) and Franke (1966) suggested they were probably due to a crossover of sympathetic fibres in the nose

In the current investigations, the vasocon striction evoked in both nasal cavities of anaesthetized cats by unilateral cervical sym pathetic stimulation has been examined at dif- ferent frequencies Experiments have also been undertaken to determine whether contra- lateral responses can be evoked on stimula tion of the opposite Vidian nerve, which con- veys post ganglionic sympathetic vasocon strictor and preganglionic parasympathetic vasodilator and secretory fibres to the nose (Slome, 1955, Malcomson, 1959, Eccles & Wilson, 1973) Some sympathetic fibres reach the nasal cavity in the ethmoidal and posterior nasal nerves (Wilson & Yates, 1977) and to in vestigate whether they are involved in the con tralateral response, vasoconstriction produced in the sympathectomized nasal cavity by stimulating the opposite cervical sympathetic chain was examined before and after section ing these nerves

Some of these findings have already been demonstrated to the Physiological Society (Wilson & Yates 1975)

METHOD

Cats of either sex 2–4 kg body weight were anaesthetized with pentobarbitone sodium 40 mg/kg I P and the trachea was cannulated

Vasoconstriction and vasodilation in the nose were recorded as a decrease or increase in pressure respectively in the sealed nasal cavity by means of a short plastic cannula

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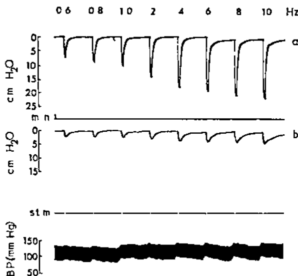


Fig 1 Vasoconstriction in (*a*) ipsilateral (*b*) contralateral nasal cavity on supramaximal unilateral preganglionic cervical stimulation at different frequencies Lower record shows arterial blood pressure

which was inserted into the nostril and connected to a pressure transducer (Bell & Howell type 4 327-L223) and a Devices M2 pen recorder (Wilson & Yates, 1975) This method only records the overall vascular changes in the nose and according to Anggård & Edwall (1974) and Malm (1974) it will main reflect the changes in the capacitance ...sels When recordings were made simulta ...sly from both nasal cavities it was ensured that a pressure change in the recording system of one side did not produce a similar change in that of the opposite side

In one series of experiments the cut peripheral end of the preganglionic cervical sympathetic chain of one side was mounted on bipolar platinum electrodes and covered with small cotton wool plugs soaked in liquid paraffin (B P) The opposite preganglionic cervical sympathetic chain was also sectioned and the ends crushed

In another series of experiments the Vidian nerve was exposed in the orbit of one side (Eccles & Wilson, 1973) The cut peripheral end was placed on bipolar platinum electrodes and covered with a small cotton wool swab soaked in liquid paraffin (B P) The posterior nasal and ethmoidal nerves were also ... in the orbit The maxillary nerve the palatine nerves and both cervical sympathe... chains were sectioned

Square wave impulses were delivered ... a Grass S4 stimulator through an isolation ... (SIU4) The preganglionic cervical sym... thetic chain was stimulated supramaxim... using a pulse width of 0 5 msec The nerve was stimulated using a pulse width 1 0 msec at an intensity of 7 0 V (Eccles & W... son, 1974) In all experiments the stimulus ... quency ranged from 0 1–30 Hz and the were stimulated for 15 sec every 3 min

In some experiments the superior ganglion of one side was removed under as... tic technique 10–14 days previously

In all experiments the arterial blood p... sure was recorded from a femoral artery means of a pressure transducer (Bell & type 4 327-221) an a pen recorder (Devi... M2)

RESULTS

Vascular responses evoked in both nasal cavities by preganglionic stimulation of the cervical sympathetic chain

The preganglionic sympathetic chain ... stimulated on one side in 10 cats at frequenc... ranging from 0 1–30 Hz and vasoconstricti... recorded simultaneously from both na... cavities

Vasoconstriction appeared quickly ... simultaneously on both sides and increas... with increasing stimulation frequency ... maximum value being attained a few seco... after stimulation began The minimal effect frequency on both sides was 0 1 Hz Maxi... vasoconstriction occurred between 10 and ... Hz Fig 1 shows the responses obtained i... typical experiment The contralateral ...sponses were, however, much smaller (F... 1*b*) and at each frequency were between ... and 20% of the ipsilateral responses (Fig 1 ... Vasoconstriction was often accompanied b... rise in arterial blood pressure at the hig... frequencies (Fig 1)

Vasoconstriction in the contralateral nasal cavity still occurred in 2 cats after acute bilateral adrenalectomy

Since the Vidian nerve generally conveys a large proportion of the post-ganglionic sympathetic fibres to the nasal cavity, this nerve was also stimulated to determine whether a vasoconstrictor response could be evoked in the contralateral cavity

Nasal vascular responses evoked by stimulating the cut peripheral end of the Vidian nerve

The cut peripheral end of the Vidian nerve was stimulated in 4 cats The vasoconstriction evoked at different frequencies was recorded from the ipsilateral nasal cavity with the contralateral cavity open anteriorly Responses to these frequencies were then recorded from the contralateral cavity with the ipsilateral cavity open anteriorly

A vasoconstrictor frequency/response curve of one of these experiments which is typical of the others is shown in Fig 2 At each frequency the ipsilateral responses have been expressed as a percentage of the maximum value observed in each cat and the contralateral responses as a percentage of the maximum ipsilateral response in the same cat The minimal effective frequency in the ipsilateral cavity was 0 1 Hz, vasoconstriction increased with increasing stimulation frequency, reached 80% of maximum at 3 Hz and the maximum value at 15 Hz In the contralateral nasal cavity, the minimum effective frequency was 0 2 Hz, the vasoconstrictor responses reaching 80% maximum at 2–4 Hz and the maximum value at 10 Hz, they were only approximately 7–15% of those observed in the ipsilateral cavity

Since the posterior nasal nerve conveys sympathetic nerves to the nasal cavity, attempts were made to stimulate it as it leaves the sphenopalatine ganglion to enter the [illegible] foramen but these proved impossible because in all the animals used, the [illegible] was too short for the application of

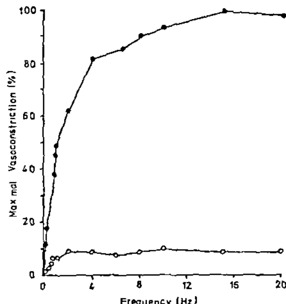


Fig 2 Vasoconstrictor frequency/response curves from ipsilateral (●—●) and contralateral (○—○) nasal cavity on supramaximal unilateral Vidian nerve stimulation in one cat One nasal cavity open anteriorly during recording from the other Ipsilateral response expressed as % of maximal vasoconstriction contralateral responses as % of maximal ipsilateral response

electrodes In no instance did Vidian nerve stimulation cause any change in arterial blood pressure

Vascular responses in sympathectomized nasal cavity

Degeneration of the sympathetic fibres was confirmed by the finding that stimulation of the cut peripheral end of the Vidian nerve on the sympathectomized side in 7 cats produced vasodilation but not vasoconstriction as in normal animals

Stimulation of the opposite preganglionic cervical sympathetic chain caused vasoconstriction in the sympathetically denervated nasal cavity In these experiments the innervated nasal cavity was open anteriorly and posteriorly

The mean vasoconstrictor frequency/response curve obtained in 7 cats, together with standard error bars is shown in Fig 3 These recordings were made at a higher

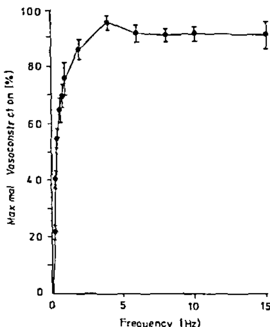


Fig 3 Vasoconstrictor frequency/response curve with S E bars from sympathectomized nasal cavity (10–14 days after removing superior cervical ganglion) on supramaximal stimulation of preganglionic cervical sympathetic chain of opposite side Innervated cavity open anteriorly and posteriorly Each value is the mean of responses in 7 cats Responses expressed as % of maximal vasoconstriction in each cat

degree of amplification than the contralateral responses shown in Fig 2 The minimum effective frequency was 0 1 Hz the responses approximately 80% of maximum at 2 Hz and maximum at 4 Hz

Vasoconstriction still occurred when the posterior nasal nerve on the denervated side was sectioned between the sphenopalatine ganglion and the sphenopalatine foramen It was reduced however by approximately 60% after sectioning of this nerve on the side of stimulation in one experiment and by cutting the ethmoidal nerve on this side in another cat

DISCUSSION

The present investigations confirm that when the preganglionic cervical sympathetic chain of one side is stimulated vasoconstriction occurs simultaneously in both nasal cavities and that it increases with increasing stimulation frequency Vasoconstriction in the con lateral cavity is however only approxim 15–20% of that in the ipsilateral cavity I unlikely for two reasons that the pressur duction in the contralateral cavity is due that caused by vasoconstriction in the ca on the stimulated side Firstly because i ensured that in the absence of nerve s tion, a pressure change in one cavity affect that in the other, and secondly it occurred on Vidian nerve stimulation w the ipsilateral cavity was open to the anteriorly

Since vasoconstriction in the ipsi and c tralateral cavities occurred simultaneou and because vasoconstriction in the con lateral cavity still occurred after acute lateral adrenalectomy, it is unlikely to be to catecholamine release Malm (1973) also of the opinion that the contralateral sponse was not due to a vasoactive agent p ing the heart because the increased pale and the decrease in venous blood flow f the nose occurred in both nasal cavitie about the same time

Post ganglionic vasoconstrictor sympath nerves are known to reach the nasal culature of the cat mainly by the Vidian subsequently the posterior nasal nerve some also reach it via the ethmoidal a bra of the ophthalmic nerve, and also by bl vessels (Wilson & Yates 1977) The occ rence of vasoconstriction in both cavities unilateral Vidian nerve stimulation sugg that the sympathetic supply to the vascula of the contralateral cavity arises peripher the Vidian nerve, probably from the sphe palatine ganglion or the posterior nasal ne The posterior nasal nerve was however short to apply electrodes to determine whe it contained sympathetic fibres for both n cavities

Vasoconstriction in the contralateral n cavity is not due to changes in arterial b pressure because in contrast to the exp ments in which the preganglionic cerv sympathetic chain was stimulated no incre

...t blood pressure was observed on Vidian ...rve stimulation

The finding that vasoconstriction was ...voked in the sympathectomised cavity by ...imulating the cervical sympathetic chain of ...e opposite side further suggests that each ...ervical sympathetic chain may convey some ...ores to the opposite nasal cavity, or that ...iose going to the cavity of the same side ...ovide to send some fibres to the contralateral ...avity Since vasoconstriction in the denerv...ed cavity is reduced by section of either ...e posterior nasal or ethmoidal nerve on the ...de of stimulation but not by section of the ...osterior nasal nerve on the side of denerva...on it would suggest that some sympathetic ...bres to the contralateral cavity arise from ...e innervated side The failure to completely ...bolish vasoconstriction by sectioning these ...erves suggests that some fibres may be con...eyed to the contralateral cavity by blood ...essels

Anggård & Densert (1974) found no evi...ence in the nose of the cat of fluorescent sym...athetic fibres crossing the midline and no ef...ect was seen on the exchange vessels on con...ralateral sympathetic stimulation This lack of ...ontralateral response is in contrast to the ...hinomanometric studies of Malm (1973) and ...he present findings Änggård & Densert ...ointed out, however, that because a relative ...lifference in the degree of the vasoconstrictor ...esponse exists between the exchange and ...apacitance vessels in the nasal mucosa (Äng...ård & Edwall, 1974) a vasoconstrictor re...ponse would most likely appear in rhino...manometric measurements which mainly re...lect the capacitance function of the nasal ...mucosa

Franke (1966) has also suggested that the ...contralateral response he observed in dogs ...may be due to sympathetic fibres of one side ...nnervating a blood vessel which supplies ...both nasal cavities Malm (1974) obtained ...ome evidence of vascular connections be...tween the nasal cavities in cats and Abe & ...Jackson (1972), using a radioactive micro-sphere technique in dogs, found a collateral circulation in the contralateral nasal cavity that was 30% of the flow in the ipsilateral cavity Änggård & Densert (1974) suggested that if a collateral circulation does exist, a leakage of adrenergic transmitter to the blood stream might explain the variance between their results and those of Malm (1973) Since in our studies the ipsilateral and contralateral responses appear simultaneously this would appear unlikely

The present findings suggest that sympathetic fibres reach the contralateral cavity by way of the posterior nasal or ethmoidal nerves or periarterial plexus of the opposite side From the studies of Abe & Jackson (1972), Anggård & Densert (1974) and Malm (1974) it is, however, more likely that the sympathetic fibres from these pathways innervate blood vessels which supply both nasal cavities

ZUSAMMENFASSUNG

Unilaterale praganglionare Reizung des Halssympathikus bei der narkotisierten Katze hatte eine frequenzabhangige Vasokonstriktion in den beiden Nasenhöhlen zur Folge Die Vasokonstriktion in der kontralateralen Nasenhohle betrug zwischen 15 und 20% der Vasokonstriktion die in der Nasenhöhle der gereizten Seite ausgelost wurde Ahnliche Ergebnisse wurden durch unilaterale Reizung des Nervus Vidianus erzielt Da die in der sympathektomisierten Nasenhohle durch Reizung des gegenuber liegenden Halssympathikus hervorgerufene Vasokonstriktion nach Durchschneidung des Nervus posterior nasalis und des Nervus ethmoidalis auf der gereizten Seite nur reduziert wird aber nicht total verschwindet darf davon ausgegangen werden daß die vasokonstriktorischen Fasern zur kontralateralen Nasenhohle entweder uber diese Nerven oder die periarteriellen Geflechte ziehen Es ist jedoch hochstwahrscheinlich daß die in diesen Nerven verlaufenden sympathischen Fasern Blutgefäße innervieren die die beiden Nasenhöhlen versorgen

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COMPARATIVE MEASUREMENTS OF THE MUCOSAL BLOOD FLOW IN THE HUMAN MAXILLARY SINUS BY PLETHYSMOGRAPHY AND BY XENON

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bstract Two different methods one plethysmographic ıd one where the elimination of radioactive inert gas as measured have been used to calculate the blood ow in the mucosa of the maxillary sinus in living man total of 5 persons were investigated and the mean values f the blood flow in the two methods showed a close orrelation Plethysmography is more easily performed nd is therefore the preferable method to be used in ud es on the physiology of the maxillary sinus

hysiological studies on the gas exchange in he maxillary sinus and studies on the function f the maxillary ostium have made it necessary o find methods for quantitative determination f the blood flow in the antral mucosa

One method for measuring the blood flow n the mucosa was reported by Drettner & Aust in 1974 This was a plethysmographic nethod which gave quantitative measure nents of the antral blood flow even if all errors of the method were not known

To be able to evaluate the reliability of the plethysmographic method, experiments were performed in which the elimination of radioactive xenon from maxillary sinuses with artificially obstructed ostia in healthy humans was measured in the same way as Elner & Nilsen (1970) did in the middle ear From the recordings of the xenon elimination the blood flow in the antral mucosa could be calculated

The two methods compared were (1) plethysmography of the mucosa of the maxillary sinus, and (2) measurements of the elimination of radioactive xenon from the maxillary sinus of living man

METHOD I
PLETHYSMOGRAPHY

In manometrical investigations of maxillary sinuses with occluded or partially occluded ostia a pulse syncrone pressure wave has been registered which can be produced experimentally in healthy sinuses in which the maxillary ostium has been closed with a tampon The magnitude of the pulse wave can be measured via a calibration which is performed by recording the pressure decrease in the measuring system caused by aspiration of a known volume of air from the system and by using Boyle s law (Drettner & Aust, 1974) The height of the pulse wave reflects the blood added to the mucosa with each heart beat, it will not show the total blood flow, however

Such a blood flow measurement can be performed by compression of the jugular veins with two fingers on each side of the neck, thus blocking most of the venous blood flow from

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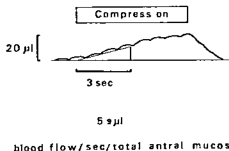


Fig 1 Plethysmography of the mucosa in the maxillary sinus during bilateral compression of the jugular veins The maxillary ostium was blocked by a tampon in the middle meatus

the viscerocranial part of the head During this compression the pressure in the investigated sinus rises and the slope of the pressure recording gives the increase in blood volume in the antral mucosa per second, thus showing the antral blood flow in the whole mucosa of the sinus (Fig 1)

The volume of the maxillary sinus and the surface area of the mucosa of the maxillary sinus can be calculated according to a roentgenological method elaborated earlier (Aust & Helmius 1974) and allows us to calculate the blood flow per surface area of the antral mu ›a We have not been able to measure the ~kness of the mucosa and have therefore the value of 125 µm, a measure reported by Loring & Tenney (1973)

By using this value together with the results of the plethysmographic and roentgenologic measurements of the maxillary mucosa and by transforming the results to a common expression for blood flow a mean value of the blood flow of the antral mucosa of 1 25 ml min^{-1} and ml^{-1} mucosa was obtained in 7 subjects in a series of investigations performed in 1974 (Drettner & Aust 1974)

The reliability of the method has been questioned, however, since the jugular veins have no valves and the measured values may thus be caused only by a retrograde progression of the increase in venous pressure to the maxillary sinus Anastomoses with other venous systems may contribute another error To be able to test the reliability of the rel... easily performed plethysmographic me... for measuring the blood flow of the max... mucosa the following method was develop...

METHOD 2
RADIOACTIVE XENON ELIMINATIO...

When looking for another principle of ... ing blood flow we found that measurement ... the elimination of radioactive xenon h... used in studies of the mucosal blood flow ... the middle ear and cell system of the mast... process by Elner & Nilsen (1970) In th... experiments quantitative calculations of t... blood flow could not be performed as th... could not measure the volume or surface a... of the examined mucosa In our experim... it was possible with the roentgenograp... method (Aust & Helmius, 1974) to calcul... the volume of the examined organ the ma... lary sinus, and the surface area of the an... mucosa Using the thickness of the ... ported by Loring & Tenney (1973) the vol... of the mucosa of the sinus could also be e... mated

A NaJ(Tl) crystal detector with a record... unit was placed in front of the sinus and ... gas elimination was recorded In the ... ments, ^{133}Xe with a half life of 5 3 days ... used A gas volume corresponding to 8 ... was injected into the sinus

It was assumed that no diffusion of xenon ... surrounding tissue took place once the bl... had left the mucosa and before the bl... reached the lungs, where all xenon was ... lieved to be immediately and totally elimin... with the expired air

Under these assumptions the following ... pression for the blood flow in the mucos... easily derived

$$\frac{V_s \ \ln 2}{V_M \ T_{\frac{1}{2}} \ K} \ \text{ml/min ml}$$

where

V_s =sinus volume (ml)

V_M =mucosal volume (ml)

t =the time to get half of the initial activity (min)
=partition coefficient, the distribution of xenon between gaseous state and soft tissue

MATERIAL

In the comparative study on the methods for measurement of the blood flow in the mucosa of the maxillary sinus, 5 healthy persons (3 women and 2 men), ages 27 to 48, were investigated Another person had to be discounted because of difficulties in occluding his ostium Further, one experiment was performed on a cadaver

Procedure of the experiments

In the healthy subjects used in the investigation the experiments were initiated by occluding the ostium with a tampon moistened with Terracortril ointment®

After the experimental closure of the ostium a cannula connected to an electromanometer with an amplifier and a recorder was introduced into the maxillary sinus through the lower nasal meatus and was kept there throughout the experiment

When the closure of the ostium was adequate, a pulse wave was registered in the antrum Insufficient closure was revealed by respiratory variations in the pressure recording, which necessitated a better closure with the tampon

The first part of the experiment was a plethysmographic measurement of the blood flow of the antral mucosa A bilateral blocking of the venous blood flow in the internal and external jugular veins by digital pressure on both sides of the neck as earlier described was performed and the mean value of these measurements was used Calibration by pressure recording during aspiration of a known small volume from the maxillary sinus was performed and the volume of the measuring system was measured in a similar procedure

Table I *Results of blood flow measurements ($ml \cdot 100\ cm^{-3}\ min^{-1}$) of the antral mucosa by plethysmography (twice) and ^{133}Xe in five subjects*

Case no	I	II	III	IV	V	Mean
Plethysmography I	61	115	46	122	98	88
Xenon	91	106	40	104	124	93
Plethysmography II	130	127	71	57	53	87

The measurements were performed with the subject in a semi-recumbent position

The second part of the experiment was started by placing the subject in a semi recumbent position with fixed head and the NaJ-crystal detector in optimal position in front of the investigated sinus

One ml xenon containing eight μCi ^{133}Xe was introduced into the sinus through the cannula which was thereafter blocked The elimination of the xenon was registered with the NaJ-crystal detector and this crystal was connected to a scaler and a semi logaritmic recorder The recording took one to two hours

After the measurement of the xenon elimination the investigated sinus was washed out with air, after which a new plethysmography was obtained in the same way as before the radioactive measurement Each person in the experiment was radiographically investigated for determination of antral volume and mucosal surface area

RESULTS

As shown in Table I the plethysmographic measurements prior to the xenon experiments gave a mean blood flow in the antral mucosa of 0 88 ml min^{-1} ml^{-1} mucosa and, after the radioactive gas experiment, a mean blood flow of 0 87 ml min^{-1} ml^{-1} The mean blood flow in the mucosa of maxillary sinus calculated from the measurements of the elimination of radioactive xenon from the antrum gave a mean value of 0 93 ml min^{-1} ml^{-1} mucosal tissue

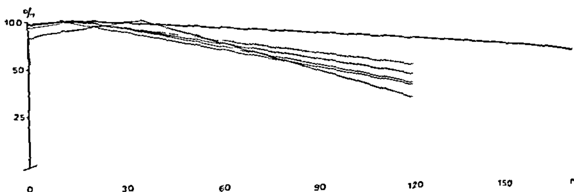


Fig 2 Recording of radioactivity from the maxillary sinus after introduction of ^{133}Xe in the sinus with a tampon occluding the ostium Values obtained in 5 subjects The interrupted line illustrates the mean value of the investigated subjects

As seen in Table I there were variations in the three measurements performed in each person but the results were of the same magnitude The variations, especially in the later plethysmographic measurements, were probably due to leakage and/or irritation As seen in Fig 2, the recordings of the elimination of radioactive xenon started with a slight increase in activity and a decrease was recorded first after about 15–20 minutes

Experiments for testing the method

The background to the initial increase of ac- y was investigated separately It was to be due to a slow distribution of gas i the maxillary sinus A similar phenomenon was reported by Elner & Nilsen (1970) in their experiments in the middle ear

A test experiment was performed w vertically placed glass tube, closed at ends, with a length of 200 mm and a diam of 15 mm in which 1 ml of radioactive x was injected in the bottom of the tube A crystal detector was placed over the top of the glass tube, and the experiment sh that the gas introduced at the lower end tube required 40 minutes to reach a con level at the NaJ detector The density Xe gas is 5 30 kg/m³ and air 1 17 kg 30°C and 101 1 kPa

The same increase in activity was rec in a cadaver experiment in the maxillary with its ostium blocked with paraffin This increase took place during the fi minutes After that the radioactivity w most constant (Fig 3)

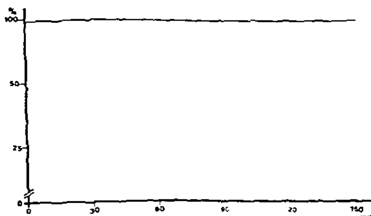


Fig 3 Recording of radioactivity maxillary sinus in a cadaver Exper ly occluded ostium

DISCUSSION

rom these last two experiments it has been own that the distribution of radioactive enon in an air filled, closed space is slow and at there is no elimination of xenon from an ccluded maxillary sinus lacking all blood ow in the antral wall There is of course dif usion of gas into the walls, but the solubility f xenon in tissue is very low (sex times lower han that of nitrogen) and this diffusion can nly have a marginal effect on the measure nents

The two methods presented for measuring he blood flow of the mucosa of the maxillary inus are the first two methods ever presented or quantitative determination of the blood low in any mucosal membrane in living man This has been made possible by our methods or calculating the volume of the sinus and the mucosal surface area The only parameter which is not known in the two presented methods is the thickness of the mucosa However if the thickness of the mucosa is assumed to be constant throughout the experiment it will not cause any error when the blood flow is calculated from the same surface area in both methods If the thickness is assumed to be 125 μm, the mean antral mucosal blood flows measured with plethysmography in the 5 investigated subjects were, before and after the xenon experiments, 0 88 ml min^{-1} ml^{-1} and 0 87 ml min^{-1} ml^{-1} respectively The blood flow calculated from the elimination of ^{133}Xe from the maxillary sinus was 0 93 ml min^{-1} ml^{-1}

A close correlation was found between the results of the two methods for blood flow measurement in the mucosa The methods attack the problem from two entirely different angles Since the results were of similar magnitude with the two methods it seems likely that both methods can be used for measurement of the mucosal blood flow in the maxillary sinus The plethysmographic method is experimentally easier and does not involve any radioactive irradiation, which facts render it the first method of choice The variations in the values are probably principally due to the difficulty in achieving a reliable occlusion of the ostium lasting as long as required when the xenon measurements are included The effectiveness of the vein compression is an other factor which can vary

The measurements gave rather high values of blood flow in the antral mucosa The same magnitude of blood flow has been reported in the nasal mucosa of cat by Malm (1974), 1 30 ml per g nasal mucosa and minute, and by Anggård (1974) 1 20 ml Different techniques were used in these two studies and none was similar to those described in this paper

ZUSAMMENFASSUNG

Zur Schatzung der Mukosadurchblutung der Kieferhohle am lebenden Menschen wurden zwei verschiedene Methoden angewandt eine plethysmographische und eine andere mit Messung der Ausscheidung inerten radioaktiven Gases Insgesamt 5 Personen wurden untersucht und die Durchschnittswerte der Durchblutung zeigten in der Anwendung beider Methoden gute Korrelation Die Plethysmographie ist leichter durchzufuhren und deshalb die vorzuziehende Methode fur Studien der Physiologie der Kieferhohlen

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THE ROLE OF LOCAL GAS COMPOSITION IN PATHOGENESIS OF MAXILLARY SINUS EMPYEMA

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Abstract An impaired ostial function may be of importance in the pathogenesis of the maxillary sinus empyema due to changes of the antral gas composition Oxygen is usually not demonstrable in purulent sinus secretion while carbon dioxide accumulates In vitro pneumococci were able to create a similar gas environment provided that the gas exchange between the medium and the atmospheric air was reduced It is suggested that heavy antral growth of facultative anaerobes such as pneumococci is related to the antral ventilation which when impeded facilitates bacterial growth

onset and course of bacterial infections e dependent on the virulence of the pathogenic microorganisms the host defence and on local conditions with regard to essential nutrients and gas composition

In purulent maxillary sinus secretions the pO_2 usually was about zero and the pCO_2 frequently above 13 3 kPa (100 mmHg), in contrast to the gas composition in cases with non purulent sinusitis as previously demonstrated (Carenfelt & Lundberg 1970) Pneumococci and *H influenzae* were most frequently isolated from the secretions

The present work was done to evaluate if the gas composition in purulent maxillary sinus secretion has any influence on the growth of pneumococci and if the bacteria may influence the gas composition

MATERIAL AND METHODS

Patients

Sinus secretion samples were aspirat through a Lichtwitz needle introduced und the inferior concha from untreated patie with symptoms of maxillary sinusitis of longer than 30 day duration Immediately af aspiration the secretions were classified purulent or non purulent (Carenfelt & l berg, 1977) Twenty seven secretions were signated as purulent and 16 as non purul All non purulent secretions in this study w of serous character In all 43 secretions fr 43 patients were collected and examined bacterial growth Twelve purulent and serous secretions were examined for p pCO_2, pH and number of bacteria and infl matory cells The bacterial number in 5 pu lent secretions was also determined in vi count Five secretions of each variety w used in air exposure experiments

Gas composition

The secretions were examined for pO_2 pCO_2 using an Ultra Micro Gas Analyser 113 Instrumentation Laboratory Inc U as previous described (Carenfelt & Lundb

77) Before the analyses the electrode sembly was calibrated with gases of known mposition, the actual atmospheric pressure d the vapour pressure of water being taken to account

-cteriological methods

imple of sinus secretion aspirated into a -rınge was transferred to a transport tube ntaining plain agar and to bottles, one for robic and one for anaerobic growth, contain g proteose peptone yeast extract with so- um polyanetholsulfonate obtained from the lational Bacteriological Laboratory, Stock olm, Sweden The materials were brought the laboratory usually within 2-3 hours and lways within 12 hours and cultured on blood gar plates (aerobic and anaerobic), blood gar with gentian violet (anaerobic), phenyl thanol agar (Difco), endoagar and hematin gar plates The plates were incubated at 37°C nd read after 24 and 48 hours The hematin gar plates were incubated in 5% CO_2 The anaerobic culture bottles were read daily either until growth occurred or for 10 days after which time subculturing on anaerobic blood agar plates was performed GasPak BBL, Cockville, M d, USA) was used for anaerobic cultivation Pneumococci, *H nfluenzae* and aerobic bacteria were identified according to conventional methods adopted by the Department of Clinical Microbiology, Karolinska Sjukhuset Isolated bacteria were freezed and stored at −70°C for later sub culturing and classification (National Bacterio- logical Laboratory, Stockholm) The anaero bic culture system, including the special equip ment for subculturing (Belco Glas Inc , Vine- land, N J , USA) developed by the Anaerobe Laboratory, Virginia Polytechnic Institute and State University (Holdeman & Moore, 1973) was used for subculturing

Quantification of bacteria and inflammatory cells

The numbers of bacteria and inflammatory cells in the sinus secretion samples were esti mated by counting in a Burker chamber Bacterial numbers less than 1×10^6 per ml and cellular numbers less than 1×10^4 per ml could not be counted with accuracy The viability of inflammatory cells was determined by stain ing with trypan blue Smears were stained in May–Grunewald–Giemsa for differential count The samples were examined immediately or stored at +4°C until reading took place within one hour of aspiration In 5 purulent secretions from which pneumococci were isolated, also viable counts by plating were done Tenfold dilutions of secretions in saline were plated on blood agar and incubated aerobically and anaerobically

Air exposure

The effect of air exposure on the gas composi tion in sinus secretions was studied by pouring 1 5 ml of the newly aspirated secretion into a glass bowl providing an air contact area of 5 0 cm^2 The secretions were exposed to atmospheric air at a temperature of 37°C The pO_2 and the pCO_2 of the secretions was determined after 0, 1, 3 and 5 min The number of bacteria and inflammatory cells in these secretions was within the range given in Table I

In vitro studies

A bacterial suspension from an overnight culture of Diplococcus pneumoniae type 3 was prepared by inoculating 2–30×10^6 bacteria per ml in a volume of 100 ml of pre-warmed fresh Todd Hewitt broth The suspension was poured either into flasks (Erlenmeyer) with a volume of 1 000 ml, thus giving a large surface/volume ratio, or into bottles having a volume of 100 ml, thus completely filled The 100 ml bottles were tightly sealed with a rubber membrane maintained in position by screw caps All flasks and bottles were equipped with a magnetic stirrer and incubated at a constant temperature of 37°C The same procedure was followed in a control experi ment, except that no bacteria were added to the fresh broth Samples of the bacterial sus pension were obtained by aspiration into 2 ml

Table I *Bacteria isolated in purulent and serous secretions aspirated from 43 maxillary sinu*

No of secretions	Number of isolates				
	D pneumoniae	H influenzae	Anaerobes	Miscellaneous	No grow
Purulent 27	12	4	9	8	0
Serous 16	3	1	0	5	8

sterile syringes at 0, 2, 4 and 6 hours for analysis One further sample was taken 24 hours after inoculation The pO_2, the pCO_2 and the pH of the bacterial suspensions were analyzed by the same method as above The number of bacteria was estimated in a Burker chamber To prevent bacterial growth between sampling and calculation, a 0 1 ml formaldehyde solution 10%, was added to 0 9 ml of the bacterial suspension Three series of 5 experiments were performed In the first series the flasks were used with the magnetic stirrer started at incubation to provide a maximum gas exchange In the second, the flasks were used but with the stirrer started after 2 hours of incubation In the third series of experiments, the sealed bottles were used to ensure that no gas exchange occurred between the bacterial spension and the atmospheric air

RESULTS

Clinical studies

Bacterial growth was found in all purulent secretions and in half of the serous secretions (Table I) Pneumococci were the most frequent findings and occurred also in serous secretions Anaerobes, on the other were isolated exclusively from the purul secretions with a predominance of *Strep coccus intermedius* and *Peptostreptococ anaerobius* The bacterial number in the pu lent secretions was about 100-fold that in serous secretions, on average (Table II) T bacterial numbers determined with via counts and in the Burker chamber in 5 purul secretions with growth of pneumococci w $1\,0\times10^9$ colony forming units/ml and 3 4× bacteria/ml on average

The number of inflammatory cells in purulent secretions was about 1 000-fold t in the serous secretions, on average (Table The proportion of viable inflammatory cel the total number of nucleated cells dete mined in 10 purulent secretions averaged (23–85) per cent In smears the inflamma cells could be classified with accuracy in 1C 17 purulent secretions The neutroph granulocytes dominated and amounted to (90 5–100) per cent, on average

In the purulent secretions the pO_2 was lo 0–1 2 kPa (Table II), irrespective of the ba teria isolated, i e pneumococci *H influen* or anaerobic streptococci In contrast t mean pO_2 in the serous secretions was sign

Table II *Number of bacteria and inflammatory cells, the pO_2 (kPa) the pCO_2 (kPa) and pH 12 purulent and 11 serous sinus secretions given as mean values*

	No of bacteria	Cells	pO_2	pCO_2	pH
Purulent					
Mean	$3\,7\times10^{9}$	$3\,7\times10^{8}$	0 1	≥11 6	6 8
Range	$1\,0\times10^{9}-7\,7\times10^{9}$	$1\,0\times10^{[illegible]}-9\,3\times10^{8}$	0–1 2	5 4–≥13 3	6 1–7 6
Serous					
Mean	$<2\,4\times10^{7}$	$<4\,1\times10^{5}$	11 7	5 3	7 4
Range	$<1\,0\times10^{6}-7\,3\times10$	$<1\,0\times10^{4}$ $1\,8\times10^{6}$	7 3–16 2	2 8–8 8	7 2–7 6

Table III *Oxygen and carbon dioxide tension, kPa, in 5 purulent and 5 serous sinus secretions before and after exposure to atmospheric air*

Volume of secretion sample 1 5 ml Contact area air/secretion 5 0 cm²

—	0 min		5 min	
	pO_2	pCO_2	pO_2	pCO_2
Purulent secretions				
Mean	0	≥12 2	0	≥10 8
Range		11 8–≥13 3		6 7–≥13 3
Serous secretions				
Mean	11 1	4 4	18 1	≤2 7
Range	7 3–16 2	2 8–6 4	12 1–22 1	≤1 3–4 6

antly higher, 11 7 kPa The mean pCO_2 was significantly higher in the purulent secretions than in the serous secretions ($p<0\ 001$, Student's t test) The mean pH in the purulent secretions was slightly acid, 6 8, whereas in the serous secretions it was slightly alkaline, 4

The gas composition in 5 purulent secretions and 5 serous secretions exposed to atmospheric air is shown in Table III In the purulent secretions with a great number of bacteria and inflammatory cells, the pCO_2 decreased while the oxygen tension remained unaltered, despite 5 min air exposure In the serous secretions, on the other hand, the pO_2 increased and the pCO_2 decreased and equilibrated with the atmospheric air, or nearly so

In vitro studies

In all experiments with wide communication between air and suspension, no exponential growth appeared No important alterations of neither gas composition nor pH were found (Table IV) When the magnetic stirrer was started at 2 hours, exponential growth was established in 2 of 5 experiments (Table V) Only in these two suspensions did the pO_2 decrease to zero and the pCO_2 increase during the experiment When gas exchange was prevented between suspension and air, growth was obtained in all experiments (Table VI) The pO_2 decreased to zero and pCO_2 increased markedly after 6 hours The gas composition in control experiments without bacteria was not changed

DISCUSSION

The common pathogens in maxillary sinusitis, pneumococci and *H influenzae* are believed to have a limited capacity to overcome the defence system of the healthy mucosa The

Table IV *Gas tension, pH and bacterial number in bacterial suspension kept in open flasks at 37°C with the stirrer started at the time of inoculation*

The figures are means of 5 experiments

Incubation (h)	pO_2 (kPa)	pCO_2 (kPa)	pH	No of bacteria/ml
[illegible]	15 8	≤1 3	7 8	1 7×10⁷
[illegible]	13 9	≤1 3	7 9	–
[illegible]	15 0	≤1 3	7 9	4 1×10⁷
[illegible]	16 6	≤1 3	7 9	3 4×10⁷

Table V *Gas tension, pH and bacterial number in bacterial suspension kept in open flasks at 37°C with the stirrer started 2 hours after the inoculation*

The figures are means of 2 experiments

Incubation (h)	pO_2 (kPa)	pCO_2 (kPa)	pH	No of bacteria/ml
0	14 9	≤1 3	7 7	2 7×10⁶
2	2 3	≤1 3	7 8	–
4	0	2 7	7 1	1 1×10⁸
6	0 7	2 8	6 8	5 4×10⁸

Table VI *Gas tension, pH and bacterial number in bacterial suspension kept in closed bottles at 37°C*

The figures are means of 5 experiments

Incubation (h)	pO_2 (kPa)	pCO_2 (kPa)	pH	No of bacteria/ml
0	16 2	≤1 3	7 7	$6 0\times10^6$
2	6 9	≤1 3	7 6	$3 1\times10^7$
4	1 3	4 5	6 9	$2 8\times10^8$
6	0	≥11 3	6 2	$5 5\times10^8$

bacterial infection has to be preceded by a virus infection or other conditions causing destruction of the mucosal surface cells, tissue oedema and exudate formation Secretion, mainly serous, is produced (van Nostrand & Goodman, 1976) and retained by the occluded ostium The damaged mucosa and the secretion are considered favourable for bacterial colonization The gas composition, the essential nutrients and the capacity of the local defence system at the site of the infection are important factors for the onset and course of bacterial infections. Another important factor may be the ability of the bacteria to influence e environment

e technical procedure used for the determation of the pO_2 and pCO_2 in the sinus cretion could be expected to cause an undue admixture of air in the sample However, in a previous study (Carenfelt & Lundberg, 1977) the method was shown to give reliable results, and in this study it was further demonstrated that any ventilation of the purulent secretions can be disregarded, as changes in the gas composition of the secretions are slow even when the contact area between atmospheric air and secretion is large

The oxygen tension in antral air is mainly dependent on the patency of the ostium but also on the oxygen absorption by the mucosa (Aust, 1974) In retained purulent sinus secretion the gas composition may also be influenced by inflammatory cells and bacteria In the present study the low oxygen and high carbon dioxide tension in the purulent secretion were concomitant with large number inflammatory cells, mainly granulocytes of them viable as judged from their abili resist staining in trypan blue The granulo is not dependent on oxygen for ingestion utilizes oxygen for certain bactericidal pro ses (Nathan & Baehner, 1971) In the puru secretions with growth of anaerobic bact the anaerobic gas environment may be du the influence of inflammatory cells In purulent secretions, however, pneumococ pure culture were isolated The bacteria numerous and exceeded about 100-fold bacteria in the serous secretions The pneu cocci, which are closely related biochemic to the anaerobes, do not utilize oxygen in energy-yielding metabolism but in the thesis of hydrogen peroxide (Austrian 19

Frequently bacteria are not only able to move oxygen from the medium, but have it in order to make the environment suit for their metabolism (Hewitt, 1950) growth of pneumococci may be retarde oxygen and facilitated in anaerobic condi and by addition of carbon dioxide (Cowa Steel, 1974). The *in vitro* experiments in present study illustrate that the pneumoc were able to create a gas environment suit for their growth and similar to that found i purulent sinus secretion, provided that the exchange with the atmospheric air impeded The experiments also show that ponential growth of pneumococci did occur when the gas exchange was efficien

Flottes et al (1960) suggested that alt gas composition in the sinus may be of imp ance to the onset of sinusitis This hypot gained support when Aust & Drettner (1 found that reduced oxygen tension in antral air is associated with ostial dysfunc prevalent in patients with recurrent sinus Frederick & Braude (1974) claimed that erobic bacterial infection in chronic s suppuration may be due to poor drainage reduced antral oxygen tension

The present results further support opinion that an impaired antral ventilatio

essential for the pathogenesis of the purulent sinusitis It can be suggested that the impaired ventilation due to ostial occlusion facilitates the bacterial and the cellular influence on the gas environment in such a way that heavy growth of facultative anaerobes such as pneumococci and of anaerobic bacteria is initiated and maintained The serous sinusitis is changed into the more serious purulent sinusitis

ACKNOWLEDGEMENT

The authors wish to thank Prof Carl Erik Nord for his generous help with subculturing and identification of anaerobic bacteria

ZUSAMMENFASSUNG

Eine beeinträchtigte Funktion des Ostium kann bei der Pathogenese von eitriger Sinusitis maxillaris von Bedeutung sein da sich die Zusammensetzung des antralen Gases ändert Saurstoff ist in eitrigem Sinussekret meistens nicht vorhanden dagegen sammelt sich Kohlendioxid an Es gelang der Nachweis dass Diplococcus pneumoniae in vitro eine ähnliche Gasumgebung zu schaffen imstande ist falls der Gasaustausch zwischen dem Medium und der atmosphärischen Luft reduziert wurde Wir schlagen vor dass starker antraler Zuwachs fakultativ anaerobischer Bakterien beispielsweise Pneumococci von der antralen Ventilation abhängig ist Wenn diese behindert ist wird der Bakterienzuwachs erleichtert

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EXPERIENCE WITH PREOPERATIVE IRRADIATION IN HEAD AND NECK CANCER

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(Received February 20 1977)

Abstract Experience with 47 patients with head and neck carc noma opera ed afte ...

of the cases at operative biopsy The remaining neoplastic cells were considered to be degenerated and non vital in 14% but in 68% of the cases the histological examina tion revealed a viable neoplasm Changes in the macro scopic appearance did not correlate very well with the histological find ngs Disadvantages of the irradiation were so minimal that the authors consider the general application of preoperative radiation in the therapy of ead and neck malignancies to be the treatment of choice

operative irradiation is gaining increasing nterest in the treatment of head and neck carcinomas (e g MacComb & Fletcher, 1957, Powers & Ogura 1965, Strong et al , 1966, Biller & Ogura 1970, Goldman et al , 1970 1972, Moore et al , 1972, Virag et al , 1976) The main purpose of preoperative radio therapy is to render neoplastic cells less cap able of invading the surrounding tissues and incapable of implantation if they become distributed in the blood or lymphatic circula tion during surgical extirpation

Recommendations for the dose of pre operative irradiation and for the interval be fore operation have varied considerably The volume of the irradiated area has also varied from irradiation of the manifest tumour only to an elective irradiation which includes the regional lymphatics

It is well known that head and neck c cinomas relatively rarely and late metastas in to the lymph nodes situated outside t neck region This fortunate behaviour l been a kind of challenge for a curative tre ment of malignancies of this area and has l to routine application of neck dissection

The recording of our data was planned observe the effect of preoperative rad therapy in relation to (1) the size of the tumor (2) the microscopic characteristics of the c cinoma, (3) the operability and complicatio (4) the effect on cure or failure in the tre ment of malignant disease with at least 3 year observation period

MATERIAL AND METHODS

All 47 subjects of this series had a histolog cally verified head and neck region neoplasm There was no intentional selection of patient for this type of therapy, but preoperative irra diation was the method of choice in cases of head and neck carcinomas treated in the years 1967 to 1972 at the University Central Hospital of Turku The Radiotherapy Clinic was re sponsible for irradiation and the ENT Depart ment for the diagnosis surgical treatment and follow up observation of the patients

Clinical patient data grouped according to the site of the tumour are given in Table I

ne histological types of the neoplasms are ven in Table II

Preoperative irradiation was delivered with Cobalt unit after an individual treatment lan to the primary tumour and included the earest lymph nodes, usually the ipsilateral ub-digastric and mid jugular nodes The radiation dose was from 1750 to 6000 rad, over -8 weeks, usually 3000 rad in 3 weeks In cases the preoperative dose was higher than 000 rad In 2 cases the radiation treatment lso included the contralateral lymph nodes

The evaluation of the effect of the treatment n the size of the tumour was estimated y careful clinical examination which was requently repeated and often recorded by hotography and measuring The other judge- were, as a rule, confirmed by biopsy pecimens

The surgical procedure was performed 1–3 weeks after the preoperative radiation In 2 ases the surgeon considered his extirpation to e only palliative In 19 cases the surgery comprised a radical neck dissection, in 13 of these lymph node metastases were detected histologically without serial section investigations n 8 cases additional postoperative irradiation was applied to a total dose of 5000–6000 rad

The observation period was at least 3 years calculated from the beginning of the preoperative radiotherapy

RESULTS

1 *Effect on size of tumour*

The changes in the size of the primary tumour estimated by clinical observation are given in Table III Disappearance of the tumour was determined by inspection and palpation In cases of disappearance the operation was carried out according to the findings before irradiation The diminution in size was estimated in two classes it was termed greatly diminished when the size at the time of operation was less than half of the original size A conspicuous diminution to a size still more than half of the original size was termed significant In 2 patients, both supraglottic laryngeal carcinomas, the size of the tumour apparently grew during the radiation therapy, and was evaluated clearly larger in the operative specimen than before therapy

2 *Microscopic findings*

Surgical specimens for microscopy were available from 44 cases The microscopic findings are in Table IV in relation to the TMN class of each case In 8 cases no neoplastic cells were seen in the preoperatively irradiated tumour specimen In 6 specimens there were neoplastic cells which were considered nonvital Among these 14 patients in which the irradiation apparently had destroyed the neoplasm, as judged by microscopic findings, there were 3 cases in which by clinical judgement a persistent residual tumour was still present although diminished in size in 2 and unchanged in one case

In 30 cases (67 %) the histological examina-

Table I *Location of the primary tumours and the age of the patients when the diagnosis was established*

	Age limits (years)	Mean age	No of patients
Larynx	37–72	58 0	21
Oral cavity	41–74	62 1	17
Nasal cavity and paranasal sinuses	32–71	57 2	6
Hypopharynx	57–60	58 5	2
Parotid gland	38	38 0	1
Total	32–74	59 0	47

Table II *Histology of the primary tumour*

Epidermoid carcinoma	
well differentiated	26
moderately differentiated	13
poorly differentiated	2
Lymphoepithelial carcinoma	1
Adenoid cystic carcinoma	3
Others (ameloblastoma melanoma)	2
Total	47

Table III *The effect of preoperative irradiation on the size of the tumor (estimated by clinical evaluation at the operation)*

Tumour state at time of operation	The size of the primary tumour				
	T_1	T_2	T_3	T_4	Totals
Disappeared	3	4	1	–	8
Greatly diminished	–	5	–	–	5
Significantly diminished	2	2	4	1	9
Unchanged	1	10	10	2	23
Enlarged	–	–	1	1	2
Totals	6	21	16	4	47

tion revealed viable malignant cells in the operative specimen after preoperative irradiation Among these cases there were 2 in which—by clinical evaluation—the tumour seemed to have disappeared totally Unfortunately no histological examination of the surgical specimen was made in 3 cases

In the 41 cases of epidermoid carcinoma the effect of radiation on the size of the tumour was correlated to the histological differentiation degree (Table V) Moderately or poorly differentiated neoplasms did not diminish significantly more often (9/15) than the well erentiated ones (11/26) (χ^2=0 76, d f =1, 0 61)

3 *Operability and untoward effects*

The effect of the preoperative irradiation upon *operability* was evaluated immediately before the operation in 22 cases, a better operability was recorded for 5 cases and no apparent change in 17 cases compared with the stage at the start of the preoperative therapy In the remaining 25 cases the surgeons opinions of the effect on operability were vague but no cases with apparent deterioration of operability were recorded

The surgeons considered in 18 cases that an *increase of operative difficulties* could be attributed to the preoperative irradiation The most common disturbance was excess bleeding In half the cases radiation oedema was considered to interfere with extirpation of the tumour The appearance of operative or p[…] operative complications seemed to have [...] relation to the radiation dose The preop[…] tive radiation could be considered respons[…] for somewhat delayed healing in 7 ca[…] Fistulae developed postoperatively in 8 [...] tients, a disturbance which can also be pa[…] due to preoperative irradiation

Table IV *Histological findings after p[…] operative irradiation in operative specim[…] from the site of the primary tumour*

	No neoplastic cells discovered	Only devitalized neoplastic cells encountered	Vital neoplastic cells present	T[…]
Stage I				
T_1N_0	2	1	2	
Stage II				
T_2N_0	2	2	9	1
Stage III				
T_2N_1	1	2	2	
T_3N_0		1	8	
T_3N_1			5	
T_4N_0	1		2	
T_4N_1			1	
Stage IV				
T_1N_3			1	
T_2N_3	1			
T_3N_3	1			
Total	8	6	30	4

4 *Survival after combined therapy by preoperative irradiation and surgery*

The surgeon decided in 2 cases, both in St[…] III (T_3N_0), to make only a partial extirpatio[…]

Table V *The relation between the differ[…] tiation degree of epidermoid carcinoma [...] the response to the preoperative irradiatio[…]*

Tumour at the time of the operation	Well differentiated	Poorly or moderately differentiated	T[…]
Disappeared	7	1	[illegible]
Greatly diminished	–	4	[illegible]
Significantly diminished	4	4	[illegible]
Unchanged	15	4	[illegible]
Enlarged	–	2	[illegible]
	26	15	41

Table VI *General result of the preoperative irradiation and surgery after at least 3 years follow-up in relation to the original stage of the tumour*

	Recovery from the malignancy		Local recurrence appeared		Died with metastases but recovered from the primary neoplasm	Total
	Living	Dead	Living	Dead		
Stage I						
T_1N_0	4	–	–	–	1	5
Stage II						
T_2N_0	9	–	–	1	4[a]	14
Stage III						
T_2N_1	4	–	–	2	–	6
T_3N_0	3	2[b]	–	3[c]	2	10
T_3N_1	2	–	–	3[c]	–	5
T_4N_0	–	–	1[d]	2	–	3
T_4N_1	–	–	–	–	1	1
Stage IV						
$T\ N_3$	1	–	–	–	–	1
T_2N_3	–	–	–	–	1	1
T_3N_3	–	–	–	–	1	1
Total	23	2	1	11	10	47

[a] The cause of death for one patient other than the malignant disease
[b] The patients died 2 resp 5 years later from other disease without signs of recurrent neck neoplasm
[c] Both local recurrence and lymph node metastases
[d] The patient is still alive 9 years later and 7 years after secondary operation

he tumour in order to avoid excessive mutilation of the patient Both of these patients died from the cancer within 2 years, irrespective of additional postoperative irradiation In the remaining 45 cases the surgical procedure was evaluated radical by the surgeon As Table VI reveals, a recurrence appeared within 3 years in 12 cases and metastases in 10 additional cases either regionally or distally The recovery percentage with 3 years' follow up is thus 53% (25/47) for the whole series, 68% 13/19) for the cases of Stages I–II and 43% 12/28) for the Stages III–IV

5 *Metastases during follow-up period*

Appearance of metastases during the 3 year follow up period is given in Table VII The Table shows that metastases appeared in 7 cases of stage N_0 Surgical radical neck dissection was not performed in any of these cases The only recurrence of metastases of Stage N_1 after surgical neck dissection appeared on the contralateral side The failure to perform a radical neck dissection in combination with the extirpation of the primary tumour of N_1 patients was due in one case to unwillingness of the patient, and a delayed bilateral neck dissection could not save another patient

DISCUSSION

Complete disappearance of a clinically evident tumour as a response to irradiation has often been observed This appeared in 8 out of 47 cases of our series all of epidermoid carcinoma Six of these were well differentiated and 2 were anaplastic The incidence of disappearance in our series corresponds to that in the report of Moore et al (1972) for example On the other hand in 2 cases of our series no response at all could be observed as the tumour continued to increase in size during the preoperative radiation therapy

When the changes in size are compared with histological findings in the operative specimens the most conspicuous finding in this series is the unreliability of the disappearance of the tumour In 2 of the present cases the

Table VII *Incidence of lymph node metastases or their recurrence within 3 years of the operative irradiation and surgery*

Original N class	So far free of metastases or their recurrence: No neck dissection	So far free of metastases or their recurrence: Neck dissection performed	Secondary metastases appeared: No neck dissection	Secondary metastases appeared: Neck dissection performed	Total
N_0	20	5	7	–	32
N_1	–	9	2	1[a]	12
N_3	–	1	–	2	3
	20	15	9	3	47

[a] Metastases appeared on the opposite side of the neck which was not dissected

histological examination revealed viable neoplastic cells although the clinically recognizable tumour had responded to the radiation by total disappearance Surgery after preoperative radiation should be carried out according to the primary plan even in cases with distinct radiation response and carefully reasoned also in cases in which complete disappearance of the tumour appears as also Goldman et al (1972) and Olofsson & van Nostrand (1973) have suggested

Surgeons often blame preoperative irradiation for additional technical difficulties during surgery In the present series radiation of up to 4000 rad, occasionally even more did not cause any excessive difficulty during or after the operation in accordance with Jakobsson & Wersall (1973) and Virag et al (1976)

In the present series 20% of the N_0 cases subsequently developed regional lymph node metastases, thus indicating a probable existence of sub clinical metastases some time before they became palpable Radiation treatment to the regional lymph nodes to a dose of 5000 to 5500 rad in 7–8 weeks should be considered seriously in most cases for eradication of sub-clinical metastases as Fletcher (1972) has proposed The additional effect of the preoperative irradiation cannot be calculated from the present series without a comparitive group of patients not irradiated

The survival figures however are consistent with those of Virag et al (1976) for example, who demonstrated signific advantages of preoperative irradiation experience does not show any significant h induced by the preoperative irradiation and equally consider its favourable effect s cant

RÉSUMÉ

L expérience avec 47 patients souffrant du carcinor la tete et du cou opérés après irradiation d'un voltage de 3000 rad pendant plus de 3 semaines est portee Pour cette série la survie était 25/47 (53 cent) pour les cas du grade I–II 13/19 (68 pour cent du grade III–IV 12/48 (43 pour cent) Par biopsie o toire on ne pouvait pas trouver de cellules néoplas dans 18 pour cent Les cellules néoplasiques étaient sidérees comme dégénérées et non vitales dans 14 cent mais l examen histologique a révelé un néopl vital dans 68 pour cent Les changements à l'appar macroscopique se ne rapportaient pas très bien aux vailles histologiques Les désavantages de l'irrad etaient tellement minimaux que les auteurs cons l application générale de la radiation préoperatoire dant la thérapie du cancer de la tête et du cou co traitement de choix

ZUSAMMENFASSUNG

Erfahrungen mit praoperativer Megavoltbestrahlung otolaryngologischen Karzinomfällen werden bericht Die allgemeine 3 Jahres Heilungsrate betrug 51% die Stad en I–II jedoch 68% Bei histologischen suchungen der Operationspraparate wurden in 18% in 14% degenerierte und zerstorte Tumorzellen aber offenbar vitales Tumorgewebe gefunden der makroskopischen Befunde d h Regression Gleichbleiben des Tumors nach der präoperativen strahlung war es nicht moglich die histologischen anderungen zu beurteilen Die Autoren finden die teile dieser Art Therapie so gering daß sie eine operative Bestrahlung als Routinemethode in Head Neck Karzinomen empfehlen

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THE INFLUENCE OF CIGARETTE SMOKE ON THE PHARYNGEAL MUCOSA

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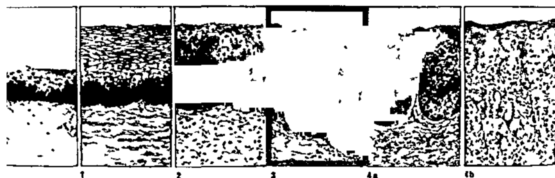
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Abstract The influence of cigarette smoke on the pharyngeal mucosa was investigated in a clinical study and in an experiment on animals Histologic reports were evaluated according to the Kambič Lenart classification of hyperplastic aberrations of the throat mucosa A close dependence of the degree of hyperplasia and the number of cigarettes smoked was revealed The more cigarettes the patients smoked every day, the more evident were changes on the mucosa more clearly manifested in male than in female smokers It was observed that the changes related to the hyperplasia atypica were more evident the longer the patients smoked They were also more intense the younger the smokers were when they started smoking In the experiment on animals a number of factors with additionally noxious influence on the pharyngeal mucosa were excluded A great interdependence was observed between the number of daily inhaled cigarettes and the changes on the pharyngeal mucosa

Various pathological changes on the pharyngeal mucosa and the number of malignancies in this region are on the increase Frequent occurrences of disease in the upper respiratory tract are associated with an altered way of life and a high standard of living and industrialization Smoking today stands accused as a significant factor in the incidence of specific diseases According to the reports of the WHO, 80% of all cancers are caused by exogenous agents or these agents contribute at least to some extent to the incidence of cancer (Silverberg & Holleb 1973) Kambic & Lenart (1968, 1971) believe that endogenous factors i e hormones, prepare favourable group for the activity of the exogenous agents and are involved in the increased risk of cancer of the larynx

Research has been conducted to determine the effect of cigarette smoke on the laryngeal mucosa in humans and in experimental animals We wanted to see whether the increased number of cigarettes smoked daily might lead to changes in the pharyngeal mucosa These changes were evaluated by the Kambič Lenart classification of hyperplastic aberrations in the mucous membrane of the throat (Fig 1) The authors divide hyperplastic changes into three grades—simple, abnormal, and atypical hyperplasia Hyperplasia simplex and hyperplasia abnormalis represent mainly the hyperplastic process on the epithelium of the laryngeal mucosa which normally does not turn malignant In a simple hyperplasia the epithelium thickens on account of the prickle cell layer The basal layer remains unchanged In subepithelial tissues very few immunocompetent cells are observed In abnormal hyperplasia, the epithelium thickens on account of the basalification Basal cells extend to the middle of the epithelium, there are no pathologic mitoses or atypias anywhere Subepithelial tissue contains more immunocompetent cells In the atypical hyperplasia cells of the entire hyperplastic epithelium have the shape of basal cells Nuclei are hyperchromatic and slightly polymorphic, here and there partially atypical mitoses appear The subepithe-

This research has been sponsored by the Slovene Research Association



· *1* The Kambič Lenart classification of hyperplastic inges on laryngeal mucosa 0 Normal epithelium 1, hyperplasia simplex, 2 hyperplasia abnormalis, 3 hyper plasia atypica 4a Ca in situ 4b, Ca invasivum

l tissue is full of immunocompetent cells, uch is significant for this aberration

By experimenting on animals we wanted to nfirm, accept, or refute our investigations in imans

METHODS

ɔirty six male and 36 female patients have :en surveyed They suffered chronic ton llitis and underwent tonsillectomy The pa ents were mostly young people, aged 24, on /erage, except for 3 who were 16 and one ho was over 50 Their occupations ranged om farm and factory workers to clerks

In 41 patients, tonsils were removed be- ıuse of frequent purulent tonsillitis Besides :current purulent tonsillitis 32 patients ıffered pain in the throat, a sense of dis omfort and of having a foreign body in the harynx during phases of the disease Three atients had peritonsillar abscesses, one had olyarthritis, one had kidney inflammation, nd one alopecia areata

After the removal of tonsils, a piece of he mucosa from the left and right frontal alatine arch was removed for histologic ex- mination The operation was in no way more omplicated by this additional procedure, nor id the patient suffer any injury, since the issue taken was that which is normally re- noved in tonsillectomy

The patients were divided into five groups, *ad modum* Ryan et al (1955), by the number of cigarettes they smoked per day

The first group consisted of those who smoked up to 9 cigarettes per day In the second group they smoked 10 to 15, in the third 16 to 20, in the fourth 21 to 34, and in the fifth, more than 35 In addition, there was a control group of non smokers

For the inhalation of cigarette smoke in animals, a special method was developed, based on the flow of air and cigarette smoke We used a special apparatus which enabled a constant apportionment of gases and the flow of the mixture through the container This procedure made a longer exposure to the cigarette smoke possible The apparatus consisted of a glass cylinder and a cover with a rubber washer on its brim and three openings through which the blend of air and cigarette smoke entered and gases from the box were re leased The third opening was used for measuring the temperature, moisture, CO, and CO in the box Cigarettes burned in a special Raucher Vulkan's device which pulsed a mixture of air and the smoke of three cigarettes into the box through a rubber tube Gases were pumped from the box by a water pump connected to the water network 42 male mice of the Albany breed, 4 months old, were used in this experiment The mice were divided into five groups of 7 and a control group which was not exposed to cigarette smoke The smoke of three cigarettes was in

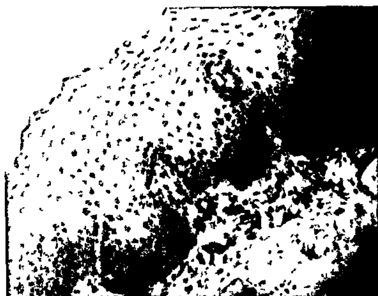


Fig 2 Pharyngeal mucosa in human. Hyperplasia simplex HE, ×255



Fig 3 Pharyngeal mucosa in human Hyperplasia abnormalis HE ×255

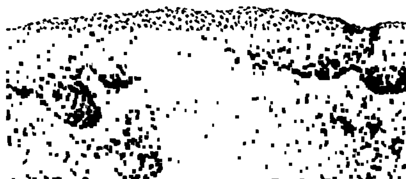


Fig 4 Pharyngeal mucosa in human Hyperplasia atypica. HE ×210

Table I *Relationship between the degree of hyperplasia and the number of cigarettes smoked daily*

Number of cigarettes	Normal	Hyperpl simplex	Hyperpl abnormalis	Hyperpl atypica
0	3	11	1	0
9	0	4	7	4
15	0	2	10	3
20	0	1	9	4
34	0	0	8	6
40	0	1	3	5

haled by the mice once to five times per day, five times a week, during 10 weeks The mice were sacrificed immediately after the last exposure In one mouse from each group, femoral vein was incised, blood for the COHb test was examined, and the pharyngeal mucosa was examined microscopically

RESULTS

In checking the first group of patients, we observed that 4 patients had signs of simple hyperplasia (Fig 2), 7 patients showed abnormal hyperplasia (Fig 3), and 4 the atypical one (Fig 4) In the second group, 2 patients had a simple, 10 the abnormal, and 3 the atypical hyperplasia

In the third group the simple hyperplasia was diagnosed in one patient, the abnormal in 9, and the atypical in 6 In the fourth group, 8 patients showed the abnormal, 6 the atypical hyperplasia In the fifth group one patient showed the simple stage of hyperplasia 3 the abnormal, and 5 the atypical one

In 3 patients from the control group the pharyngeal mucosa was healthy A simple hyperplasia was observed in 11 patients of this group and only in one patient was the abnormal stage of hyperplasia diagnosed (Table I)

The experimental mice were regularly

Table II *Relative weight increase in mice*

Group	control	1	2	3	4	5
Increase	4 4	3 9	2 2	3 2	1 6	0 7

Table III *COHb in mice blood*

Group	COHb (%)
Control	0
1	20
2	20
3	28
4	34
5	31

weighed and the relative weight increase was measured Their weight diminished when the daily number of the inhaled cigarettes increased The highest relative weight increase was observed in the mice of the control group (Table II) No COHb in blood was found in animals of the control group The COHb level increased with the number of exposures to the cigarette smoke up to 34% as in heavy smokers (Table III)

Autopsy of the mice revealed no macroscopic pathologic changes on the pharyngeal mucosa The mucous membrane was pale pink and moist In one mouse from groups 2, 3, 4 and 5, an atelectasis of lungs was observed In 2 mice from group 4, changes in the lungs were observed which were histologically diagnosed as abscesses Microscopic examination revealed that the thickness of the epithelium had decreased on account of the prickle cell layer, when the number of exposures increased With the increased number of inhalations the number of mitoses increased These were measured in ten fields of view (Table IV)

The following observations were made in separate groups

1st group=a simple hyperplasia was observed in 2 mice, atypical in one, abnormal in 4
2nd group=simple hyperplasia was seen in 2, abnormal in 4 mice, atypical in one
3rd group=simple hyperplasia developed three times, abnormal and atypical twice each
4th group=simple hyperplasia was seen in one mouse, abnormal in 4, atypical in 2



Fig. 5. Pharyngeal mucosa in mouse. Normal epithelium. HE ×255



Fig. 6. Pharyngeal mucosa in mice. Hyperplasia simplex. HE ×255



Fig. 7. Pharyngeal mucosa in mice. Hyperplasia abnormalis. HE ×255

Table IV *Number of mitoses in different groups (measured in ten fields of view)*

Group	Number of mitoses
Control	9 28
1	9 28
2	14 28
3	13 71
4	13 57
5	19 00

5th group=abnormal hyperplasia developed in 3 mice, atypical in 4

Five mice in the control group had a healthy mucosa, and 2 showed symptoms of simple hyperplasia (Figs 5–7)

DISCUSSION

In spite of the obvious influence of the cigarette smoke on the incidence of malignancies in the pharynx, it is impossible to establish its effect accurately

We are of the opinion that alcohol and hygienic and dietetic factors (Rothman & Keller, 1972, Schmidt & DeLint, 1972) are also associated with the occurrence of cancer in this region It is very difficult to determine the effect of separate agents Differences in their degree of risk render the evaluation of the effect of these factors even more complex Besides, the number of the cigarettes smoked each day is not constant, all smokers do not inhale in the same way, nor exhale the same composition of gases

In the experiment on animals these factors were not considered, and consequently only the effect of the cigarette smoke on the pharyngeal mucosa could be investigated

The basic prerequisite for the experiment on animals was the production of a human model of smoking though it was not possible to develop an ideal one

In smokers, solid particles of the cigarette smoke are arranged differently, the bulk of them remaining in the nose (Dalhamn et al, 1968) In humans, lungs are the most exposed region, in animals, fewer particles enter the lungs Almost any method of supplying cigarette smoke to animals is unnatural and even with the best technical equipment, the dose of cigarette smoke which the animal receives cannot be exactly established (Armitage et al, 1974)

We have proved, however, that cigarette smoke has a harmful effect on the pharyngeal mucosa of mice The greater was the number of cigarettes inhaled, the more severe were the changes on the mucous membrane (r=0 70, α=0 001) With the increase in daily number inhaled cigarettes, the number of mitoses rose (r=0 50, α=0 001), the epithelium grew thinner on account of the prickle cell layer and the basal layer grew

In the experiment on animals, a number of factors having an additionally noxious effect in humans, were eliminated, and consequently, our observations show only the influence of the cigarette smoke We have to consider the fact that exposures were of short duration, that the animals were sacrificed immediately after the experiment and that the corneous mucosa of the pharynx in mice is less prone to the harmful effect of smoking than is so in humans However, there is evidence that even in animals the cigarette smoke does affect the pharyngeal mucosa

CONCLUSION

In our research into the dependency between the degree of hyperplasia and the number of

Table V *Relationship between the degree of hyperplasia and the number of cigarettes inhaled daily in mice*

Number of cigarettes	Normal	Hyperpl simplex	Hyperpl abnormalis	Hyperpl atypica
0	5	2		
3		2	4	1
6		2	4	1
9		3	2	2
12		1	4	2
15			3	4

cigarettes smoked daily, the following conclusions were reached

The greater was the number of cigarettes smoked, the greater were the changes on the pharyngeal mucosa ($r=0\ 75$, $\alpha=0\ 001$) The earlier the age when smoking started, the greater the changes that occurred ($r=0\ 51$, $\alpha=0\ 001$) Our studies indicate that in male smokers, smoking the same number of cigarettes daily, the changes that fall into the atypical hyperplasia category were more frequent than in women χ^2 test for evaluating the relationship between the variables has proved significant at the 0 01 level

Differences in the degree of hyperplasia show that there are endogenous agents to be considered, as has already been pointed out by Kambič et al (1973)

ZUSAMMENFASSUNG

Der Einfluß des Zigarettenrauches auf die Rachen schleimhaut wurde in einem klinischen Experiment und im Tierversuch untersucht Die histologischen Resultate wurden nach der Klassifikation von Kambič und Lenart für die hyperplastischen Aberrationen an der Kehlkopf schleimhaut gegliedert Die Autoren haben einen großen Zusammenhang zwischen dem Schweregrad der Hyper lasie und der Zahl taglich gerauchter Zigaretten ge Je mehr Zigaretten die Patienten taglich rauchten so großer waren die Veranderungen an der Rachen leimhaut ausgepragter beim Mann als bei der Frau Der Grad der Hyperplasie war auch von der Zeit abhangig je langer die Patienten rauchten desto mehr atypische Hyperplasien wurden in den histologischen Praparaten gefunden Im Tierversuch konnten einige zu satzliche schadliche Faktoren für die Rachenschleim

[illegible]

grad der Hyperplasie der Rachenschleimhaut

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THE EARLY VASCULARIZATION OF AN AUTOGENOUS BONE INLAY INTO AN ARTIFICIAL DEFECT IN THE RABBIT MANDIBULA

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Abstract The revascularization of two different autogenous bone grafts to an artificial defect in the rabbit mandibula was studied by means of microangiography using Indian ink infusion into the carotid artery The revascularization was compared between a longitudinally split autogenous humeral full thickness bone graft in 19 rabbits and autogenous full bone grafts from the iliac crest in 14 rabbits The vascular pattern and ingrowth was followed at weekly intervals right to the end of the first month postoperatively A progressive revascularization was found in both grafts investigated The periosteum of the humeral graft was earlier and more completely revascularized than when an iliac graft was used The penetration and downgrowth of capillaries were on the other hand more complete within the iliac graft The vascular pattern is discussed with special reference to the osteogenesis in the bone grafts

The role of the vascularization as regards the osteogenesis of bone grafts has been debated in clinical as well as in experimental studies of bone transplantation Phemister showed in his basic experimental work (1914) that the viability of the cells of the transplant is largely dependent upon their ability to get nutrition He also claimed that the periosteum and the endosteum superficially located received sufficient nutrition to survive and proliferate Albee (1921) stated that an early and adequate blood supply to the graft is necessary for a favourable bone growth Stringa (1957) claimed that "there is a striking correlation between the rate of vascular penetration of the bone implants and their ultimate "take" or incorporation in the bed' Zuckman et al (1968) believed that the development of newly formed vessels is a necessary preliminary stage for osteogenesis but not sufficient in itself

The purpose of this investigation was to study the early vascularization of a split full thickness autogenous humeral respectively autogenous full thickness iliac bone graft into an artificial defect in the rabbit mandibula

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MATERIAL AND METHODS

Fifty two young adult rabbits about 6 months of different breed and sex were operated on The operations were carried out with the animals under general anesthesia maintained by intravenous injections of Mebumal® (ACO) in a dose of approximately 30–35 mg per kg body weight and inhalation of Ether ad narcosin® (Skånska Bomullskrut) Meticulous aseptic precautions were taken The right mandibula was exposed through a curved incision along the inferior border of the jaw bone and a 15×5 mm segment of the bone was cut out using a

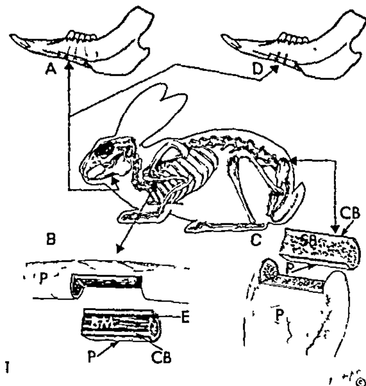


Fig 1 Operative procedure (A) [...] defect in the lower border of the max[...] Dotted line indicates the mandibular [...] (B) Longitudinally split full bone gra[ft] [...] the humerus with periosteum (*P*), c[...] bone (*CB*) endosteum (*E*) and bone [mar]row (*BM*) (C) Full bone graft from th[e] [...] crest with periosteum (*P*) thin c[...] bone (*CB*) and spongious bone (*SB*) [...] Bone inlay (from the humerus or the [...] crest) secured to the host bone by osteosutures

0 3 mm wide diamond saw-blade (Fig 1) by means of a motor-driven drill (MEDA) under continuous irrigation with sterile Ringer's solution The resected piece of bone and periosteum included the content of the mandibular canal and the apical part of the three first molar teeth with an extension from the mandibular angle to the symphysis of the mandibula In one group of 23 rabbits, a full bone autograft from the iliac crest was placed as an inlay transplant into the mandibular defect The transplant included periosteum with a thin shell of cortical bone surrounding the cancellous bone of the crest In the other group consisting of 29 rabbits, an autograft from the right diaphysis of the humerus was split longitudinally and placed together with its periosteum, endosteum and bone marrow into the defect in the jaw bone The purpose of shaping as large a graft as possible without handicapping the animals resulted in a humeral graft about 1 mm shorter in height than the graft from the iliac crest The fresh bone grafts were never kept *ex corpore* for more than 20 minutes and were during the preparation [of] two bore-holes in the host bone for the sutures kept in physiological saline solu[tion] The transplants were then secured to the gr[aft] bone by means of two 0 3 mm wide s[...] osteosutures passing through bore holes [in] the mandibula and around the transplant Th[e] incised jaw muscles and the skin were close[d] with interrupted sutures The wound was the[n] dressed with an adhesive solution of N[o]becutan® (Bofors) All animals received inje[c]tions of penicillin 1 1 million IE immediately after the operation and on the two succeeding days The animals were fed on 2-3 dl per da[y] of food pellets (Fors) with a standardized con[]tent of calories, minerals and vitamins The rabbits were sacrificed at weekly intervals ranging from 9–30 days postoperatively

METHODS OF INVESTIGATION

Microangiography

A microangiographic technique with Indi[an] ink infusion was used in the study of the [...]

,cularization of the graft On the day of rifice the animals received Heparin® (Vit-n) 10000 IE, 5 ml Xylocain® (Astra) 0 5% d an overdose of Mebumal® (ACO) intra-nously Thereafter the right commune rotid artery was exposed and cannulated in cephal direction and 300–400 ml of a 20% dian ink solution was infused during 1–1½ urs from a height of 150 cm above the heart vel of the animal, as described by Olerud & inckwardt Lilliestrom (1971)

-eparation of specimens for morphological alyses

iter the completion of the Indian ink in sion the skull was removed and the jaw one was freed from soft tissues except for a in periosteal layer surrounding the bone he two osteosutures were carefully ex-racted The transplant and surrounding host one were transversally divided into three qual parts by a specially designed saw with a 3 mm diamond blade under continuous irri-ation and under adjustable pressure One art was prepared for light microscopy, one or fluorescence microscopy and one for microangiography according to the Spalteholz chnique The parts investigated with these chniques were chosen at random and varied idividually so that one piece of a specimen ould be investigated by three different meth-ds

'reparation for light microscopy

The specimen was fixed with a solution of for-naldehyde 10% for 2 days, decalcified with richloracetic acid 3 5% for one week, em-edded in paraffin wax and cross sectioned in ~10 μm thick slices with a sliding microtome MSE) Alternating sections were stained with iematoxylin and eosin, and Mallory's Azan nethod A macrophotograph of the section was taken with a Hasselblad camera adapted or Bellows and Zeiss luminar lenses The ob ervations of histological sections were per-ormed in ordinary light microscopy and photographed with a Zeiss photomicroscope

Preparation for microangiography

After fixation with 10% formaldehyde solution and decalcification with trichloracetic acid 3 5%, the specimen was embedded in paraffin wax and cross sectioned with a diamond saw-blade using the above mentioned specially designed saw under continuous irrigation and adjustable pressure into 0 5–1 mm thick slices deparaffinized in xylol, and clarified with methylsalicylate according to the technique described by Spalteholz (1914) The Spalte-holz preparations were examined in a stereo-microscope (Zeiss) and photographed with a Hasselblad camera adapted for Bellows and Zeiss luminar lenses

RESULTS

Complications and mortality

Anesthetic complications constituted a major problem, especially initially, and it was found that a safer and deeper general anesthesia was obtained by diluting the Mebumal from 30 to 15 mg per ml and combining it with ether ad narcosin Animals dying from anesthetic complications prior to the completion of the surgical procedure were not recorded and are therefore not included in this material Opera tive complications were very rare Some ani mals where the humeral autografts exceeded more than half the circumference of the hu merus developed a fracture of the humerus but after about 3 weeks could again use the limb The blood loss during surgery was usually minimal but 3 animals developed large hematomas which had to be removed from the mandibular wound In spite of the penicillin treatment, 6 animals developed an infection in the mandibular wound Three of these animals developed so extensive an abscess formation that the healing process was seriously im-paired and thus had to be excluded from the survey

Out of 52 animals operated, 16 died during the observation period Of these, 1 died some hours after the operation probably due to an anesthetic complication or a fat embolus, 2

died of toxicity of hematoporphyrin, a bone labelling agent used for fluorescence micro scopy, 1 died for a reason unknown, and 12 died of an epidemic hemorrhagic enteritis

All animals that expired before the end of the observation period and the 3 above men tioned heavily infected animals and one animal where the angiogram was not considered rep resentative, were excluded from the investiga tion The total number of animals used for analyses was 33, of which 19 underwent an im plant from the humerus and 14 underwent an implant from the iliac crest (Table I)

The animals were unwilling to eat during the first postoperative days but could usually eat carrots or animal pellets on the third day As a rule they lost 2–3 hg during the first week but usually regained weight subsequently

Microscopical observations

A detailed report on the observations made by light and fluorescence microscopy will be given in a following publication Only data relevant to the pattern of circulation are dis cussed in this paper

MICROANGIOGRAPHY

Nine Days Postoperatively

Humeral inlay

A network of fine capillaries penetrated the gap between the graft and the host bone perpendicularly from both the medial and lateral sides though more abundantly from the medial side The vessels radiated along the endosteal side of the graft and sometimes completely filled the former medullary cavity of the grafted humerus (Fig 2) Revascularization of the disrupted apical pulp vessels had also started at that time The cortex of the graft was still avascular except in one animal where tiny capillaries pierced the cortex from both the endosteal and periosteal sides The perios teum of the graft was in all cases supplied by wide vessels, in particular on the medial side In all cases there was a good correlation be

Table I *Material of rabbits used f[illegible] phological studies*

Observation time (weeks)	Humeral inlay Histo-logy	Humeral inlay Micro-angio-graphy	Lia mbr Histo-logy
1	5	5	3
2	3	4[a]	3
3	4	4	3
4	6[b]	5	5
Number of in vestigated rabbits	18	18	14
Total number of in- vestigated rabbits	Humeral inlay 19		1

[a] Only microangiography performed in
[b] Only histology performed in one rabbit [illegible] roangiogram was not considered representative

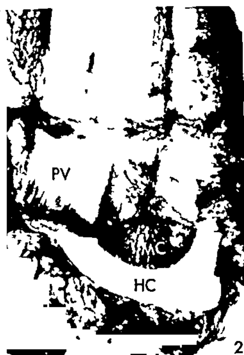
tween the formation of woven bone [illegible] vascularity (Fig 3), although [illegible] of cartilage formation and subsequent [illegible] chondral ossification on the periosteal

Iliac inlay

The ingrowth of capillaries was here [illegible] siderably sparser but followed the [illegible] tern as described above The vessels penetrated along the osteotomy line [illegible] some cases grew down some fractions [illegible] millimeter along the trabeculae of the [illegible] (Fig 4) The vascular supply to [illegible] of the graft was scanty and in some [illegible] vessels had not yet reached the periosteal [illegible] The osteogenesis was retarded in all cases [illegible] to an inadequate blood supply (Fig 5) [illegible] there were no signs of cartilage formation

Fig 2 Spalteholz preparation showing a 0 8 mm [illegible] cross section of a humeral graft inserted into the [illegible] mandibula after 1 week Note the penetration of [illegible] from the medial side (*M*) into the medullary cavity [illegible] There is a nascent revascularization of the pulp [illegible] (*PV*) while the humeral cortex (*HC*) is totally [illegible] Magnification ×12

Fig 3 Histologic preparation from the same [illegible] in Fig 2 demonstrating the periosteal callus (*PC*) [illegible] host bone invading the medullary cavity from the [illegible] side (*M*) Endochondral ossification (*EO*) is seen on [illegible] periosteal side of the humeral graft Nascent osteo[illegible] the humeral cortex (*HC*) is also seen Magnification [illegible]









4 M croang ogram showing the almost avascular il ac i *IG*) after 1 week The vessels are penetrating the otomy l ne from the medial side (*M*) and start re- cularization of pulp vessels (*PV*) The vessels have not pierced the deeper layer of the periosteum (*P*) of the t. Spalteholz preparation 0 65 mm th ck in cross sec i Magnif cat on × 15

Fig 5 Cross section of histologic preparation of the same animal as in Fig 4 with good periosteal callus (*PC*) on the med al side (*M*) of the host fragment The osteotomy line is filled with woven bone on the lateral side but in this specimen there is only granulat on tissue on the med al side The epiphyseal plate (*EP*) within the graft fragment is not closed Magnification × 10

Sixteen Days Postoperatively

Humeral inlay

The vascularization around the cortex of the graft was by now intense and the vessels were wide and tortuous They completely filled the former medullary cavity of the graft and had with slender capillaries pierced the graft cortex from both the endosteal and periosteal sides These vessels did not form an anastomosing network within the graft cortex (Fig 6) The lower third of the pulp was now occupied by wide capillaries The periosteum was also completely vascularized, but there were still signs of endochondral ossification on the periosteal side The development of trabecular bone surrounding the graft was abundant (Fig 7) and the osteotomy line was hardly visible, though the bone formation within the graft cortex was not pronounced

Iliac inlay

The vessels penetrated deeper into the graft There was a preponderance of a perpendicular downgrowth of vessels from the well vascularized gap between the graft and the host bone The vessels infrequently pierced the thin cortex from the dilated periosteal vessels of e graft The vasculature surrounding the aft was substantially less than when a humeral inlay was used, though the interior of the graft was intersected with anastomosing capillaries of varying size occupying the proximal third of the graft In one animal the whole transplant was vascularized except for the epiphyseal plate (Fig 8) The bone formation around the graft was not so intense as when a humeral inlay was used, although there was new bone laid down upon the trabeculae within the graft cortex (Fig 9) No signs of cartilage formation were seen

Twenty-three Days Postoperatively

Humeral inlay

In 2 of 4 animals an extensive circulation developed in the graft cortex The circulation in the other two graft cortices was poorer In some cases, there was a deficient filling vessels in the former medullary cavity graft This deficiency became even evident in the pulp and the sinusoidal n of the interdental spaces of the host bor 10) If this was due to intravital or post thrombi could not be determined by the graphic appearance The histological showed a good endosteal bone formati an evident resorption of the graft Th formation within the graft cortex w pronounced even here On the periosteal there was no cartilage formation

Iliac inlay

In one animal there was a poor vascular to the graft In the other two animals th was interlaced with a mesh of anastor capillaries occupying the upper two-thi the graft (Fig 11) Even here there reduction of the filling of the vessels pulp of the teeth The vessels penetrat graft in the same fashion as described ously, though in one animal the vesse traverse the cortex from the well vascul periosteum Only few vessels were se pass the epiphyseal plate Though ther an abundance of vessels in the junctio tween the graft and the host bone there mixture of dense fibrous tissue and trab bone New bone was laid down upon vascularized trabeculae inside the graf cartilage growth was observed

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Fig 6 Microangiogram from a Spalteholz prep 0 7 mm thick in cross section showing abunda cularization around the humeral graft after 2 week the good revascularization of the pulp vessels (PV nification ×12 5 The inset above shows good cularization of the intercortical capillaries w graft from both the endosteal and periosteal sides fication ×17

Fig 7 Cross section of histologic preparation f same specimen as in Fig 6 The periosteal callu the medial side (M) of the graft was accidently re when the osteosutures were taken out The me cavity (MC) is completely filled with trabecular Magnification ×9 5









Fig 8 Microangiogram from a Spalteholz preparation 8 mm thick in cross section demonstrating almost total revascularization of the iliac graft (*IG*) after 2 weeks The thin cortex (*C*) on the lateral side (*L*) is not so well vascularized and there are no vessels traversing the epiphyseal plate (*EP*) Magnification ×13

Fig 9 Cross section of histologic preparation from the same specimen as in Fig 8 Note the broad periosteal callus (*PC*) from the medial side (*M*) of the host bone No new bone formation is seen from the periosteum (*P*) of the graft Magnification ×9 5

Thirty Days Postoperatively

Humeral inlay

In 4 animals of 5 investigated there was a marked increase in the vascularity of the graft The vessels in the marrow of the grafted humerus were dilated and well filled The periosteal circulation was intense and the circulation within the cortex of the graft appeared in some cases to be as vital as the circulation in the cortex of the host bone (Fig 12) Quite often an extravasation of Indian ink into resorption cavities in the graft cortex was noticed There was a good filling of the sinusoidal interdental network and the pulp cavity was in some cases supplied to its whole extent by capillaries, though by much fewer than normally The histological picture showed an intense osteogenesis both endosteally and periosteally without cartilage formation The graft cortex had undergone advanced resorption and subsequent bone formation

Iliac inlay

In 2 of the 5 investigated animals, there was a complete filling of anastomosing vessels within the graft However, few vessels had been le to penetrate the epiphyseal plate in these ses (Fig 13) In one animal, the graft was supplied with wide, tortuous vessels in the upper half of the graft but only slender capillaries were observed further down In two specimens the graft was poorly vascularized with slender capillaries

The capillaries penetrated the graft, in the same manner as described previously, from the gap between the host bone and graft down and along the trabeculae of the graft, and very seldom did the vessels pierce the thin cortical layer from the periosteal side of the graft The histology showed often a mixture of dense fibrous tissue and newly formed bone in the gap between the graft and the host bone There was a good bone formation upon well nourished trabeculae within the graft Cartilage growth with subsequent endochondral ossification occurred in one c se only

DISCUSSION

Comments on the microangiographic method

There are various methods of visualizin
microcirculation of bone tissue Severa
vestigators have noted an insufficient v
ization of the intracortical system when
Micropaque (Gothman, 1961, Trueta 1
Danckwardt-Lilliestrom (1969) suggeste
this contrast medium during the first wee
lowing operation might have greater diff
in passing the subperiosteal bone vessels
did Indian ink Brånemark et al (1968) fo
lacking penetration of Indian ink, which
ascribed to intravascular aggregation of c
carbon Another disadvantage with the I
ink infusion method is the necessity for
thick specimens Perfusion with M
(coloured silicon rubber) maintains its p
ties during decalcification and sections
µm or less can be made (Hunsuck, 19
pilot study performed by the author com
the Microfil and Indian ink infusion techn
did not show any remarkable difference
visualization of the microvasculature
jaw bone Rubin et al (1964) have stress
importance of employing a physiologic
fusion technique Due consideration has
taken in this respect Thus, the injecti
Heparin 10000 IE reduced the occurre
post-mortem thrombi The infusions w
ways made in the direction of flow
bloodstream, and with pressures nev
ceeding that of the systolic blood pre
The duration of infusion was more tha
hour, which led to a good filling of the v
studied

Fig 10 Spalteholz preparation 1 mm thick in cro
tion showing good revascularization of the intra
vessels of a humeral graft after 3 weeks The
deficient filling of the medullary cavity (*UC*) pe
sels (*PV*) and the periodontium (*Pe*) Magnificat on
Fig 11 Spalteholz preparation 0 6 mm thick in cr
tion demonstrating capillaries growing down d
graft (*IG*) after 3 weeks There is an avascula
centrally and distally in the graft Magnification x

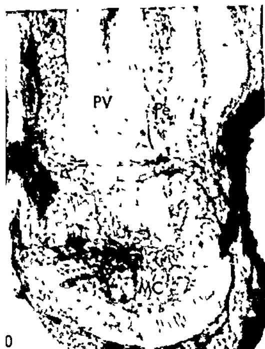








Fig 12 Spalteholz preparation 0 75 mm thick in cross section showing good revascularization of a humeral graft after 4 weeks Note the rich revascularization of the pulp vessels (*PV*) Magnification ×11 The inset above demonstrates thin capillaries piercing the graft cortex mostly from the endosteal side The arrows along the endosteal border of the graft indicate extravasation of Indian ink into resorption cavities Magnification ×17 6

Fig 13 Spalteholz preparation 0 7 mm thick in cross sec [illegible] penetrating the epiphyseal plate (*EP*) Magnification ×9

Pattern of vascularization

The present investigation shows, in accordance with other investigations, that the invasion of vessels to the graft fragment occurs from the interfragmentary region and in particular from the medial aspect This is probably due to an impaired circulation on the lateral side caused by the surgical trauma, while the circulation from the well nourished mucous membranes of the mouth remains It is also shown by this study that during the observation period there was an increasing vascularization of both a longitudinally split full thickness autogenous humeral inlay and a full thickness autogenous inlay from the iliac crest On the whole, the humeral graft became earlier and more completely surrounded by functioning vessels than the iliac graft The reasons for this are probably various

Firstly, the shape of the humeral inlay fits the defect created in the lower jaw better than does the iliac graft, which often is too narrow at its base This causes often a poor approximation to the head fragment with mobility and a subsequent tipping of the graft

Secondly, the vessels at once reach the humeral graft from all directions while the vessels from the medial side have a longer distance to go before reaching the narrow iliac graft, which often is placed in absolute contact with the host bone on the lateral side The vessels also had a longer way before reaching the most distal parts of the interior of the iliac graft It has been shown that if the marrow circulation becomes seriously impaired the original centrifugal bloodflow to the cortex to a major part will be replaced by a centripetal bloodflow from vessels in the surrounding soft tissues (Brookes et al , 1961, Rhinelander, 1968, Dankwardt-Lillieström et al 1970) In the present investigation, the main flow was in a centrifugal direction on the endosteal side of the graft but when a humeral graft was used there was in addition a considerable centripetal flow from the vessels in the enveloping soft tissues via the periosteum towards the cortex In accordance with the accepted belief, the interior of the iliac graft after four weeks was supplied by a richer network of capillaries than was the cortex of the humeral bone Obviously, the reason for this is the more open texture of the iliac graft where the vessels without any resistance can grow down and along the graft The vessels have greater difficulties to anastomose in cortical bone because they must first bore out cavities through the bone to make their way

Thirdly, the better revascularization of the periosteum of humeral grafts could possibly depend on a more abundant capillary supply of this kind of periosteum per se This might be explained by the greater functional demand upon the humerus, which in the rabbit is the chief support of the foreleg, whereas there are no such demands upon the iliac crest Phemister (1914) showed the better regenerative powers of the humeral periosteum in comparison to ulnar periosteum in dogs [illegible] et al (1972) showed the superiority of tibial periosteum as compared to the periosteum of calvarian bone in rabbits as regards the osteogenic potency Another barrier to the ingrowth of capillaries from the periosteum of an iliac graft consisted of the epiphyseal plate which rarely and then first after three to four weeks made room for vessels in either direction

In the present study there were clear signs of resorption of the humeral graft by the widening of Haversian vessels already after days, but hardly any vessels had at that time penetrated into the graft This is, according to Brookes (1971), a characteristic reaction of compact bone to medullary ischemia which can proceed without signs of any remarkable osteoclasia He suggests that the acid metabolites and protein breakdown products caused by profound ischemia provide the initial stimulus for the formation of a reparative osteogenic blastema This grows towards the ischemic area bringing with it abundant new vascular channels In the present investigation there was an increased filling of the intracortical vessels from the second week onwards when humeral grafts were used The

ar network showed the same architec- structure as the normal mandibula These support the conception that the widening Haversian vessels within the grafted facilitates the recanalization of the pre- Haversian system Albrektsson (1971) ed that structures lining the vascular ls of fresh bone grafts had ATP ase ac- at a time presumably preceding that of ılar ingrowth thus suggesting partial val of the vascular endothelium of the Early invasion of host tissue into the t by means of recanalization of pre existing graft vessels has been propounded by ral investigators (Hancox, 1947, Stringa, , Deleu & Trueta, 1965, Olerud & Dank- Lilliestrom, 1971)

enesis and vascularity

ogenesis and vascularity are closely re- which has been clearly shown by the of Trueta & Cavadias (1955) and ta (1963) Gothman (1961) found the live- arterial response related to regions where aque callus was heaviest in tibial frac- in the rabbit and monkey Maurer et al , 1965) showed that the formation of eral callus always was preceded by a of hypervascularization Zuckman et al) claimed that though the development formed vessels was a necessary pre- stage for osteogenesis, it was not suf in itself Thorogood & Craig (1975) ed through both light microscopy and microscopy that vascularization al preceded the osteogenesis in bone graft In the present study there was a strong ation between the early revasculariza of humeral graft and both bone resorption new bone formation The humeral bone early became surrounded by a dense lary network and the new bone formation ys followed in its wake As regard iliac s new bone formation growing from the line down and along the graft fol ng the same paths as the capillaries could be observed The vascularization of the gap between the graft and host bone was in all cases more pronounced when a humeral graft was used The development of trabecular bone was accordingly more decided in these cases thus resulting in a better bony attachment of the graft to the host bone The iliac grafts were, in fact, united to the host bone by a mixture of dense fibrous tissue and cancellous bone

Cartilage formation

Cartilage formation often occurs in fracture repair and bone transplantation The extent to which this occurs, or why it occurs at all, is still an unsolved problem Leriche & Policard (1926), Urist & McLean (1941) claimed that cartilage develops in fractures as a result of inadequate immobilization Ham (1930) and Girgis & Pritchard (1958) found cartilage formation in fracture repair when the blood supply was insufficient Reddi & Huggins (1975) have described the transformation of fibroblasts to chondroblasts with subsequent endochondral ossification as the vascularity increases In the present investigation, there were signs of cartilage formation with subsequent endochondral ossification in almost all humeral grafts during the two first weeks This occurred exclusively on the periosteal side This reaction subsided after two weeks and was not seen in any case after 3 weeks Neither cartilage nor endochondral ossifica tion was to be seen when an iliac graft was used, except in one case which occurred after 4 weeks Thus the explanation for cartilage formation with endochondral bone formation could not be imperfect immobilization, because there were never any signs of displacement of the humeral graft Even though the blood supply in this group seemed adequate as judged from the angiographic appearance, the callus formation on the periosteal side grew so rapidly that the blood vessels could not keep up with the osteogenic cells which under these circumstances became transformed into chon droblasts This is in accordance with theory proposed by Ham & Harris (1956).

more difficult to explain why there was no cartilage formation when iliac grafts were used The retarded revascularization in combination with a paucity of osteogenic cells in this kind of periosteum might be one of the probable causes of this phenomenon

There are many methods of improving the blood circulation to a free bone graft Baadsgaard & Medgyesi (1965), Baadsgaard (1970), Medgyesi (1973) found improved and more rapid revascularization by using a bone graft with its vascular supply mediated through a muscle pedicle Strauch, Bloomberg and Levin (1971) have explored the possibility of using a vascular island composite rib graft to the mandibula in dogs They reported good survival of the bone graft and patency of the supplying blood vessels Recently, Ostrup (1975) showed good survival of a composite rib graft in dogs by means of a microvascular anastomose between the intercostal and lingual vessels Though the results from that investigation appear very encouraging, the method is laborious and takes a well trained and skilful surgeon to perform this operation As shown from the present investigation, a ne graft implanted into well vascularized ues will rapidly receive a blood supply netrating into the bone marrow, while this ppens more sluggish in the Haversian system of a compact bone The choice of method for the clinical work will have to take into consideration the need for well vascularized tissue for bone transplantation This is achieved in many flap techniques though the results vary (Millard et al 1967 Millard et al 1970 Conley, 1972 Conley et al 1973) There might, however, be some cases where the surrounding tissues are sclerotic or else poorly vascularized where an intact circulation or microvascular anastomoses might give better results than free bone grafts

CONCLUSIONS

1 There was a progressive revascularization of both a split full bone autogenous humeral graft and a full bone autogenous iliac g[...] when implanted into an artificial defect [...] rabbit mandibula

2 The new capillaries arising from ves[...] in the soft tissues enveloping the jaw bo[...] vaded the graft during the first weeks ma[...] through the interfragmentary region and [...] ponderantly from the medial side

3 The periosteum of the humeral graft [...] earlier and more completely revascular[...] than when an iliac graft was used

4 From the second week onwards [...] periosteal vessels supplied the outer [...] cortical vessels of the humeral graft to an [...]creasing extent, while they could hardly pe[...]trate the epiphyseal plate of the iliac graft.

5 The penetration and downgrowth [...] capillaries were after 4 weeks more comp[...] within the marrow of the iliac graft than wi[...] the humeral graft, which on the other hand [...]came earlier and more completely surroun[...] by functioning vessels

6 There was a good correlation betw[...] vascularization and osteogenesis within [...] around the grafts

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ZUSAMMENFASSUNG

Die Revascularisierung in zwei verschiedenen Arte[...] Knochentransplantaten eingesetzt in einen artif[...] Schaden im Unterkiefer von Kaninchen wurde mit [...] einer mikroangiographischen Methode untersucht [...] Tusche in Arteria carotis infundiert wurde Der Gra[...] Revascularisierung wurde zwischen einem au[...] Transplantat vom Humerus längs gespalten in 10 F[...] und einem autogenen Vollknochentransplantat v[...] Crista ilica in 14 Fällen verglichen Das Mus er de[...] fasseinwachsens wurde Woche fur Woche bis [...] ersten Monats nach der Operation kontrolliert [...] eine progressive Revascularisierung in beiden Arte[...]

splantat Die Periost des Humerustransplantates le fruher und mehr vollstandig als die Periost des atransplantates revascularisiert Dagegen war die tration und das Herunterwachsen von Gefassen voll 1 ger in dem spongiosen Teil des Cristatransplantats Einwachsen von Gefassen wurde mit spezieller sicht auf die Knochenneubildung in und rund der hiedenen Arten von Transplantaten diskutiert

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CYTOLYTIC ACTIVITY OF TONSIL CELLS

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ract The ability of normal canine tonsil cells and of humans with chronic tonsillitis to lyse heterorocytes in vitro is shown Heterologous erythrocyte uction by tonsil cells is markedly increased after im zation of the animal Tonsil cells of patients with nic tonsillitis lyse the erythrocytes coated with tococcus and staphylococcus antigens more actively xperimental tonsillitis increases the ability of tonsils se erythrocytes Since cytolytic activity of tonsil is not altered by the removal of phagocytes and treat with antiglobulin serum one can presume that it is ed by T lymphocytes

966 Oettgren et al showed that the pala tonsils of patients with chronic tonsillitis tain 40% of lymphocytes, which can be in reted as thymus derived cells because of r ability to transform into blasts being in nced by phytohemagglutinin This fact was her confirmed by the determination of phocytes able to form rosettes with sheep blood cells (Brown & Greaves, 1974, evitch, 1975) and with the help of specific serum (Greaves & Brown, 1974) The pres of the cells' thymic origin in animal s ton in experiments on thymectomized rabbits zuki & Iho, 1973) and dogs (Buchem & jpers 1973) has been established The fact T lymphocytes are lymphoid system ef or cells prompts the suggestion that tonsils an active part in cellular immunity reac s (Gjulling 1972) Nowadays the ability of nan and animal tonsil lymphocytes to cause t vs host reactions is accepted (Harrison, 1972, Nikolsky et al , 1975) It has been shown that a considerable proportion (up to one-third) of cells react by transformation to blast cells with streptococcus and staphylococcus antigens, in populations of small lymphocytes *in tonsils of patients with chronic tonsillitis* (Vizirenko & Gorshevikova, 1972, Grinevitch & Vizirenko 1973) It has also been demonstrated that the migration inhibitory factor is released from tonsil lymphocytes of patients with chronic tonsillitis under the influence of microbic antigens (Rocklin et al , 1972, Melnikov, 1973)

We have studied the cytolytic activity of tonsil lymphocytes that is, the most important index of cellular immunity tension The results of the determination of the cells capacity for non specific and immune cytolysis in the normal and inflamed state are given in the present report

MATERIALS AND METHODS

In 22 mongrel dogs (1–4 years old, weight 5–15 kg) cytolytic activity of tonsil cells was studied in intact dogs and animals immunized with 10^7 hen erythrocytes, injected into tonsils

0 2 ml of mixture consisting of equal parts *of complete Freund s adjuvant (Calbiochem)* and a 24 hour culture of β haemolytic strepto coccus and containing about 2 billion microbic bodies per 0 1 ml was injected into tonsils of 3

dogs to produce tonsillitis within one hour after injection of the heteroerythrocytes

Six dogs of another group received the infective mixture twice The second injection was given 2–3 weeks after the first These dogs were immunized within a month of the second injection Tonsils of experimental dogs were extracted 10–11 days after immunization

Cytolytic activity of tonsil cells from patients with chronic tonsillitis was evaluated according to the results of the non-specific destruction of hen erythrocytes and to the index of cytolysis of erythrocytes coated with staphylococcus and streptococcus antigens In *the latter case, cytolysis may be a specific* characteristic, as the microbes mentioned are believed to induce tonsillitis

Cell suspensions of tonsils from 49 patients, 15–50 years of age and suffering from chronic tonsillitis for 5–8 years, were used

Excised tonsils were washed in Hanks' solution with antibiotics (penicillin and streptomycin, 100 U/ml), cut very finely, and passed through a nylon filter into siliconized tubes

The cell suspension was washed twice with Hanks' solution at 4°C by centrifugation (150 *g*, 3 min) and finally suspended to a concen-on of 5×10^7 cells/ml in the following um 87 ml of medium 199, 10 ml of fetal serum inactivated by heating, 100 fold concentration of L-glutamine, amino acids and vitamins per 1 ml, penicillin 100 U/ml, streptomycin 60 γ/ml

A portion of tonsil cells was treated with carbonyl ferrum in order to remove phagocytes from the suspension in a magnetic field by the method of Kupers et al (1961) Another phagocyte-depleted portion of tonsil cells was incubated with complement and human globulin antiserum at 37°C for an hour in order to block B cells After that the cells were washed in tissue culture medium twice for 3 minutes at 150 *g*

Erythrocytes of chicks not more than 4 months old were used as target cells Erythrocytes obtained in sterile conditions were conserved in 0 85% sodium chloride solution with

Table I *Cytolytic activity of tonsil cells fr[illegible] intact dogs and from dogs immunized [illegible] chicken erythrocytes*

Statistical indexes	% Isotope (^{51}CR) release from target cells — Groups I	II	III	IV
M	3 5	11 1	25 0	24
±*m*	1 9	1 6	5 8	5 2
n	8	6	3	5
P	–	–	<0 05	<0 05

I intact animals, II normal immunized dogs III d[illegible] immunized before induction of tonsillitis IY–dogs [illegible]munized after second induction of tonsillitis

25 U/ml heparin for up to 48 hours Ta[illegible] treated hen erythrocytes were coated [illegible] complete streptococcus and staphylococ[illegible] antigens obtained by the method of Versh[illegible] (1969) These erythrocytes were aggluti[illegible] with homologous serum (titres 1 16-1[illegible] in a passive haemagglutination react[illegible] Erythrocytolysis induced by lymphocytes [illegible] determined according to the recommenda[illegible] of Perlmann et al (1970)

The basic experiment was conducted in [illegible]tral glass bottles by adding chicken eryth[illegible]cytes (10^6) to a lymphoid cell suspension [illegible] in the ratio 10 · 1 and a constant Volume [illegible] medium of 1 ml

Killed lymphocytes, or 2×10^7 non iso[illegible] labelled chicken erythrocytes, were added [illegible] control bottles The bottles were incubated [illegible] 24 hours at 37°C and the percentage of [illegible] release as regards the spontaneous release [illegible] isotope in controls was determined accor[illegible] to the method of Perlmann et al (1970)

The results were evaluated by Student's test

RESULTS

The data presented here indicate the abil[illegible] normal dog tonsils and those of people [illegible] chronic tonsillitis to lyse heteroerythro[illegible] *in vitro* (Tables I, II) After immunizat[illegible]

mals with heterologous erythrocytes, de ction of the latter by tonsil cells increases rkedly (Tables I, II) Tonsil cells of ients with chronic tonsillitis lyse more ac ly the erythrocytes coated with strepto cus and staphylococcus antigens (Table An experimental tonsillitis increases tonsils' capacity for erythrocytolysis ble I)

e removal and treatment of phagocytes h antiglobulin serum does not adequately ct the cytolysis of heteroerythrocytes by sil cells

DISCUSSION

non specific destruction of various target ls by lymphocytes has already been re ted and recognized as a manifestation of phenomenon of allogenic inhibition utsui & Everett, 1974, Hayward, 1974) or on relevant' killing (Willumsen & Heron 4) Erythrocytolysis brought about by ton ells is probably similar to or identical with phenomenon described By its nature, an quate increase in the cytolytic activity of sil lymphocytes caused by preliminary im nization of dogs by target cells, is evidence the tonsils capacity for cellular immunity ctions

An increased destruction of heteroerythro es coated with microbic antigens by tonsil phocytes in patients with chronic tonsillitis not be explained as being a result of their osure to the above mentioned microbic cimens, as no increase in spontaneous lysis he treated erythrocytes is noted in the con ls The cytolytic effect is not associated h phagocytosis of target cells either since gocyte removal does not affect the level of throcytolysis As patients with chronic ton itis almost always have antibodies to ptococcus and staphylococcus one can y consider the destruction of erythrocytes ited with antigens to these microbes to be only the result of immune cytolysis caused the sensitized lymphocytes but also the

Table II *Cytolytic activity of tonsill cells in presence of chronic tonsillitis*

Statistical indexes	% Isotope (^{5} Cr) release from target cells Groups I	II	III	IV	V
M	10 5	25 4	23 6	27 2	35 6
±*m*	1 4	3 6	4 1	2 6	[illegible] 0
n	39	37	38	10	5
P	–	<0 05	<0 05	<0 05	<0 05

I chicken erythrocytes + tonsil cells II chicken erythrocytes coated with staphylococcus antigens III chicken erythrocytes coated with streptococcus antigens + tonsil cells IV chicken erythrocytes coated with streptococcus antigens + tonsil cells without phagocytes V chicken erythrocytes coated with streptococcus antigens + treated with antiglobulinum serum tonsil cells without phagocytes

product of cytolytic activity of cells with mem brane receptors to Fc fragments of IgG

Treatment of tonsil cell suspension with an tiglobulin serum capable of blocking B cells (Resch et al 1974) does not suppress the erythrocytolysis This fact permits us to con sider the T lymphocyte to be mainly an ef fector cell

The increase in erythrocytolysis in the pres ence of chronic tonsillitis may be caused by an accumulation of lymphoid cells presumably T population in the focus of inflammation (Fedotov 1969 Asherson & Alwood 1973)

ZUSAMMENFASSUNG

Die Zellen der Gaumenmandel von gesunden Hunden und der Leute mit Tonsillitis konnen die heterologischen Erythrozyten (Test-Cr[51]) in vitro zerstoren Nach der Immunisierung der Tiere war die cytolitische Aktivitat viel großer Die Bearbeitung der Erythrozyten von Kucken mit den ant genen Streptococcus haemolyticus oder Staphylococcus aureus hat Bedeutung und zur Zerstorung solcher Erythrozyten geführt Die experimentelle Entzundung in den Tonsillen hat die cytolitische Aktivitat erhoht Die Entfernung der Phagozyten im magne tischen Feld und auch die Bearbeitung der Lymphozyten aus Tonsillen von dem Serum gegen menschliche Globulinen hatten die cytolitische Aktivitat nicht verandert Nach der Meinung der Autoren wird diese Aktivitat durch T Lymphozyten hervorgerufen

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PERIPHERAL VASOCONSTRICTION IN THE RAT IN RESPONSE TO SOUND

I *Dependence on Stimulus Duration*

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r Vasoconstriction in response to sound has been in the non-anaesthetized rat Arterial pulsations tail were recorded by a non invasive technique band noise bursts at 80 dB SPL with durations 1 ms to several hours were presented in a free a sound isolated box The results show that energy is integrated with a time constant of about The responses during continuous stimulation ha ed slowly and the time to halfway normalization of pulsation was more than 15 min

tail of the rat has a rich vascular supply ' serves an important function in thermo- lation (Rand et al , 1965) The blood flow vary more than one hundredfold in re e to different demands on heat dissipa- (Johansen 1962) When room tempera is normal, the tail arteries are constricted the flow is low Above an ambient tem of about 30°C the artery dilates and ws heat to be lost When dilated, sensory uli of various types, especially a drop in ıre but also sound and painful stimuli elicit constriction of the vessels in the tail ome basic characteristics of the acoustical- licited vasoconstriction of the rat's tail ves have recently been described, together a technique allowing repeated recordings his reflex in the non anaesthetized rat • 1977) The blood flow in the tail artery indirectly assessed in terms of amplitude lsations recorded from the surface of the A short burst of broad-band noise was found capable of eliciting vasoconstrictions at sound levels down to the threshold of hearing The degree of vascular reaction, measured as the duration of the response, increased approximately linearly as a function of sound level, at least over 80 dB (sound pressure level, re 20 μPa) The latency time was about one second, and the total duration of the response exceeded one minute With presentation intervals of 10–15 minutes there was insignificant habituation over an 8–10 h period for 80 dB SPL noise bursts This acoustic-vascular reflex was suggested as a suitable model for studies of influence of sound on autonomic functions

The aim of the present study is to determine how the vascular response varies as a function of the duration of a broad band noise signal of 80 dB SPL

Two experiments were performed (*a*) temporal integration was investigated for short noise bursts, 1–4 000 ms, (*b*) adaptation, or habituation of the vasoconstriction was determined during prolonged sound exposure

MATERIAL AND METHODS

The experiments were performed on 13 adult male Sprague-Dawley rats Five of them were

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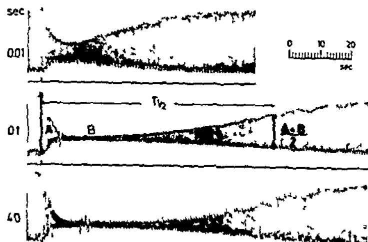


Fig 1 Recordings of arterial pu[...] from the tail of a non-anaesthetized ra[...] 80 dB SPL noise burst elicits a vaso[...]striction quantified by $T_{½}$ time from s[...]lus onset until pulse amplitude is h[...] normalized Stimulus duration (10 [...] 4 000 ms) is indicated below each tr[...]

experimentally naive, the remainder had been subjected to similar experiments earlier The measurements were separated in two sessions, 10 of the animals participating in both of them

The technique has been described in detail earlier (Borg, 1977) and only some essentials will be outlined here During the experimental session the animal rested in an individually adjustable net tube on a regulated heating pad The blood flow in the tail was assessed in terms of pulsations recorded by a rubber balloon connected to a volume-sensitive trans- (Elema 510C) Sound stimuli were pre- ' through a Lancing L 75 loudspeaker 10 in front of the rat and consisted of 80 dB SPL broad band noise bursts of varying d[...]tion The maximum energy was in the ra[...] 3–15 kHz (see figure 2 in Borg 1977) and [...] rise and decay times of the bursts were [...] 1 ms The bursts were presented with appro[...]mately 15 min intervals and each experim[...] session lasted between 8 and 12 hours

The duration of the noise burst was 1 [...] 20, 50, 100, 1 000, or 4 000 ms in the first [...]perimental session Each burst was presen[...] several times in random order In the seco[...] experiment, some weeks later, bursts of lo[...] duration, namely 1, 10, 100 and 990 s w[...] presented at random and finally a continu[...] noise was introduced In this latter sessio[...]

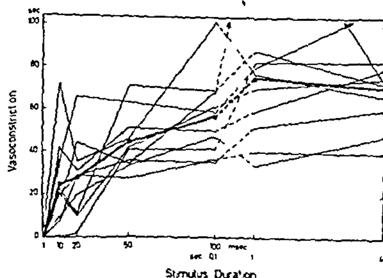


Fig 2 Duration of vasoconstri[...] ($T_{½}$) as a function of duration of 8[...] SPL noise bursts in 10 rats Each [...] line represents one experiment Hea[...] line shows average Abscissa is d[...] with two scales Two values fall outs[...] frame

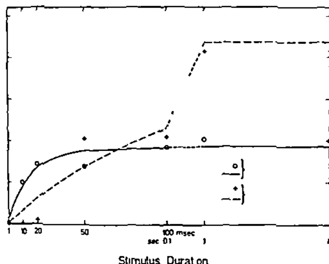


Fig 3 Observed durations of vasoconstriction in response to 80 dB SPL noise bursts of different duration for 2 animals (+ and 0) and the adapted exponential function $y = a[1-\exp-(1/Tx)]$ where a is the asymptote and T is time constant The fitness was 60% and 87% and the estimated T was 125 and 16 ms respectively

e or a few presentations were made at setting The vasoconstriction was quan- as the duration of the response from the of the sound until the amplitude of the ation had returned halfway to the pre ulus level (T_1 in Fig 1)

imuli were presented only when certain na were fulfilled the pulse recording had e free from artifacts indicating ongoing or activity of the rat, responses were dis- ed if the initial vasoconstriction was rseded by subsequent spontaneous re- ses before the pulse amplitude had re- ed halfway to pre-stimulus level (see fur- Borg 1977) Less than 10% of the re- nses had to be discarded for that reason in first experiment, and none in the second eriment

RESULTS

Response to short noise bursts

ordings of arterial pulsations obtained the surface of the tail of the rat are shown Fig 1 which illustrates typical effects of dB SPL noise bursts of different durations 100, 4000 ms) About 1 s after the onset he noise burst (indicated below each re ding) the pulse amplitude declined After ut 1 min it had recovered completely As a rule, a 10 ms noise burst elicited a smaller and shorter reaction than bursts in the range of 100–4000 ms

The duration of vasoconstrictions in response to stimuli of different lengths is shown in Fig 2 as thin continuous lines for the 10 individual rats The heavier line shows estimated mean values It is seen that the individual variability is considerable, but generally the short sounds elicited smaller responses than sounds of 50–100 ms duration The average curve more clearly illustrates the temporal integration in the vasoconstriction reflex

In order to obtain an estimate, at least a

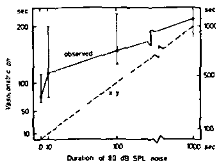


Fig 4 Duration of vasoconstriction in response to extended 80 dB SPL noise bursts median and semi inter quartile range (±25%) Each point represents 12 rats Broken line shows hypothetic situation without adaptation response being equal to stimulus Left ordinate corresponds to stimulus duration 1 10 and 100 s and right ordinate to 990 s bursts

rough one, of the time constant for temporal integration in this reflex, each individual set of values was described by an exponential function, $R=a[1-\exp-(1/Tx)]$, where R is response duration, a corresponds to the horizontal asymptote, T is the time constant, and X is duration of stimulus The parameters were determined by a non linear (Gaussian) process In 5 of the animals the fitness was better than 60% and the time constant varied between 16 and 125 ms, averaging 60 ms (median 50 ms) The time constant of the system is then approximately 0 1 s

Fig 3 illustrates the adapted functions and the observed values for 2 animals The fitness was in these cases 60% and 87% respectively, and the time constants were 125 and 16 ms

B *Response to extended noise*

The duration of the vasoconstriction elicited by a long stimulus, from the beginning of the sound to the moment the pulse amplitude first exceeded (A+B)/2, is illustrated in Fig 4 During prolonged stimulation (100 990 s) the pulse amplitude often waxed and waned, exhibiting a sequence of vasoconstrictions Such multiphasic responses have been excluded in the nalysis of short bursts (Figs 1 and 2, and rg 1977) For the sake of consistency those and 10 s bursts followed by multiple constrictions were not excluded in Fig 4 and the values for 1 (and for 10 s) are not directly comparable with the values for 1 and 4 s of Fig 2

The median and semi interquartile range from 12 experiments in 12 rats are illustrated The broken line shows the duration expected to occur, should the system be completely without adaptation i e duration of stimulus and response are equal Responses to stimuli up to 100 s did not adapt Several individual values were on the other hand considerably larger than expected and so was the median value During 990 s stimulation the response habituated in 5 cases, after 122 to 207 s but mostly it outlasted the noise considerably

On continuous stimulation the pulse amplitude returned halfway to pre-stimulus value after, on the average, 16 min (median 1[illegible] stable vasodilation was, however, not re[illegible] until after 47 min (median range 25 [illegible] min) It is also to be noted that the con[illegible] noise was presented after several stimuli[illegible] range 1 to 990 s Rate of habituation [illegible] therefore be overestimated in these e[illegible] ments compared with habituation to a [illegible] tinuous "novel stimulus At any ra[illegible] evident that even with a fairly short pe[illegible] rest between presentations, responses only slowly habituating

DISCUSSION

The present results show that 1) noise [illegible] with durations less than approximately [illegible] are relatively inefficient for the product[illegible] vasoconstriction, 2) the vasoconstrictio[illegible] bituates, but slowly, when the rat is e[illegible] to continuous 80 dB noise

The variability in the responses is con[illegible] ble (Figs 2 and 3) and this technique is [illegible] be compared with many psycho-ac[illegible] methods with respect to reliability of [illegible] measured value In an earlier study [illegible] 1977) the influence on the responses of s[illegible] factors was analysed The effect of vari[illegible] in temperature and body movements is [illegible] crease variability without introducing sy[illegible] atic errors, since stimuli are presented r[illegible] dom order

It is interesting, though, to observe th[illegible] time constant for integration of acoustic [illegible] gy in this acoustic–vascular reflex is [illegible] same order of magnitude as time consta[illegible] loudness (e g Zwislocki, 1969) It rema[illegible] be settled whether the similarity of ti[illegible] stants signifies a functional relationsh[illegible] tween acoustic perception and sound-el[illegible] autonomic activation A possible morph[illegible] cal substrate for such an interaction in t[illegible] is already well established the haben[illegible] gion of the modulus in the inner ear (S[illegible] lin & Lichtensteiger, 1966, Densert 19[illegible]

In the present experiments several [illegible] longed noise presentations were made

short intervals (about 15 min), yet these only poorly adapting responses It lier been shown (Borg, 1977) that this does not habituate to 1 s bursts of 80 dB noise presented with 10–15 min interval at least 8–12 h A peripheral vasocon- n in response to sound has long been in humans (e g Mosso, 1874, Jan- 1974) and is stable during prolonged ex- (Lehmann & Tamm, 1956) Although endurability of the vasoconstriction may sound has a permanent influence cardiovascular system, it is premature ate about harmful effects of environ- tal sound other than hearing loss

ZUSAMMENFASSUNG

ichtanasthetisierten Ratten wurde die Gefäßkontrak als Antwort auf Tongeräusche untersucht und zwar Aufzeichnung der arteriellen Pulsationen im mittels einer nichtinvasiven Methode Als Sti dienten hoherfrequente breitbandige Gerauschstöße pakete von einer Starke von 80 dB SPL und un lichen Dauern von 1 ms bis zu mehreren Stun im freien Schallfeld in einer Schallbox Das Resultat e daß die akustische Energie integriert wird mit Ze tkonstante von etwa 0 1 s Die Antwort auf kon erliche Stimulation habituierte nur langsam und die bis zum Amplitudenanstieg der arteriellen ationen halbwegs auf den Ausgangswert betrug mehr 5 min

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TRANSTYMPANIC ELECTROCOCHLEOGRAPHY DURING GLYCEROL DEHYDRATION

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Abstract Thirteen patients with Meniere s disorder were tested using transtympanic electrocochleography during glycerol dehydration The most common finding was a decrease in the negative summating potential and this appeared to be a more sensitive indicator of changes occurring in the cochlea than pure tone audiometry and speech discrimination A pathophysiological explanation for this observation is offered and the possibility of using this decrease in the negative summating potential as a pointer to the prognosis of endolymphatic sac surgery is discussed

The diagnosis and treatment of Meniere's dis- nas both fascinated and frustrated physi- for many years Diagnosis may be dif- because many conditions including syphilis, vertebro-basilar insufficiency, cochlear otosclerosis, perilymph fistula and acoustic neuroma may be characterised by Meniere's syndrome Meniere's disorder is an idiopathic condition and can only be diagnosed after all the known causes of the syndrome have been properly excluded Patients with an incomplete symptom complex are often diagnosed as Meniere's disorder in the belief that they represent early or even atypical forms of the disease but a proportion of these patients are inevitably incorrectly diagnosed The assessment is also complicated by certain well known non-organic factors which have an association with the disorder namely personality type and the response of the patient to environmental stress A useful tech which appears to confirm the diagnos many cases is transtympanic electrococ graphy (Gibson et al , 1976)

PATHOGENESIS

Apart from the diagnostic problems the considerable difficulties in the managem these patients The long list of medica surgical treatments for Meniere's disord flects our inadequate knowledge of the p genesis Hallpike & Cairns (1938) first de strated the presence or endolymphatic drops in post mortem studies and this has largely confirmed by subsequent worker have attempted to correlate pathol changes with ante mortem symptoms (knecht, 1963) The aetiology of the hydr not known but many attractive theories been proposed which are either conc with the production, reabsorption and ci tion of the endolymph or with an alterat the endolymph potential secondary to ch in ATP activated ion balance

Spasm of the stria vascularis has attra great deal of attention It may interfere endolymph circulation, thus producin drops Disorders of the endolymphatic sa duct have also been incriminated The m

is endolymphaticus may be an important . endolymph reabsorption or may act as compensating for changes in endo- ' pressure Malfunction may then lead olymphatic hydrops

ly, doubt has been cast on the theory ymph actually circulates and it may the stria vascularis acts as an ATP lled electrotonic mechanism, which ns the ionic composition and resting al potential of the endolymph It is pos- that no circulation occurs except in conditions when the saccus endo- may act as an important site of re- on and as a compensatory chamber i of the stria vascularis may render the media anoxic, leading to a progressive ease in sodium and decrease in potassium n the endolymph, altering the endolymph ntial from approximately +100 mV to mV (Bosher & Warren, 1968), and per- s leading to hydrops There may be a criti level of endolymph potential at which the tae of the semicircular canals lose their ing neural function causing an attack of nal vertigo Distension of the scala could become so great as to rupture ssner's membrane, causing an ion inter- nge and sudden alteration in the endo- ph potential This would result in a more longed attack of vertigo Unfortunately, nal studies give conflicting results in dif- species and there is no animal model of s disorder, although endolymphatic ,, with a low frequency fluctuating ring loss, has been demonstrated in me- cal cochlear models by Tonndorf (1968)

GLYCEROL DEHYDRATION TEST

a trivalent alcohol (1,2,3, propane- l) given in high doses is not metabolized letely and is an osmotic diuretic, being ' in the urine Klockhoff & Lindblom) used glycerol dehydration to produce icant hearing threshold shifts in cases of ere s disease with fluctuating hearing loss They presumed that the osmotic effects of the glycerol reduced the endolymphatic hydrops and intra labyrinthine pressure and this was confirmed by Bosher & Warren (1971) Klockhoff & Lindblom employed 1 5 g/kg body weight of glycerol, although more recently they have used 1 2 g/kg Originally, increases in the pure tone threshold of 5 dB were taken as significant, but more recently, a positive result has been taken as at least a 10 dB improvement in three adjacent octave bands, or speech discrimination improvement exceeding 12% (Angelborg et al , 1973) No effect was seen in more advanced Meniere's cases where a non fluctuating flat loss was present, or in sensori neural deafness of less specific types (Klockhoff & Lindblom, 1966) Morrison (1975), however, observed that in patients with well established Meniere's disorder there may be a post glycerol improvement in speech discrimination in the absence of a pure tone threshold change In a small proportion of patients there may even be an unexplained deterioration in the pure tone threshold or in speech discrimination in response to glycerol dehydration (Klockoff, 1975)

More recently Snyder (1971), confirmed Klockhoff's findings but felt a 15 dB increase in the pure tone threshold was more likely to be significant He found that the changes were more marked in the low tones and also noted a significant increase in the speech discrimination Shea (quoted by Klockhoff, 1975) suggested that a good response to glycerol dehydration was an indicator of a better prognosis in endolymphatic sac surgery

This paper explains the role of continuous transtympanic electrocochleographic recording during glycerol dehydration in studying the electrophysiological changes in the cochlea

METHOD

Transtympanic electrocochleography, as described by Gibson et al (1967), was employed Adult patients were tested without sedation

using the method of iontophoresis to anaesthetise the tympanic membrane (Ramsden et al, 1977)

Prior to the electrocochleography, blood and urine samples were collected for osmolality estimations A pure tone audiogram and an assessment of speech discrimination were also carried out All patients were starved from midnight for 12 hours before the test

The electrodes were positioned with the patient lying comfortably in the supine position A pre glycerol recording was carefully made and particular attention paid to electrode contact and the consistency of the response Iced, lemon flavoured glycerol, 1 2 g/kg body weight, was given to the patient via a pliable straw so that the fluid could be taken without moving the head position The immediate post glycerol response was then checked and full recordings to clicks and frequency specific sine waves at 1 and 2 hours post glycerol were made The patients were also monitored at more frequent time intervals In 2 patients the recordings were terminated at 1¾ hours and at 1 hour respectively, because the patients became uncomfortable lying in one position for so long

paratus

e Medelec Amplaid Mk III ERA equipment was used This apparatus is simple to operate and is suitable for clinical purposes The detailed specifications may be obtained on request from the manufacturers In this study, the calculations in the tables and appendix are based on the responses obtained using acoustic clicks of 100 μs electrical duration Spectral analysis of the click produced from the earphone showed that it contained a wide spectrum of frequencies with its maximal acoustic energy at 3 kHz Pure tone bursts of 2–8 sine waves at specific audiometric frequencies were also employed as stimuli

The stimuli were presented whenever possible, from a mu metal screened earphone which was attached magnetically to a special ring support placed around the ear Occasionally, the transducer was a loudspeak positioned 50 cm from the test ear Th situation was avoided whenever poss since the test chamber was not anechoic T bandpass of the differential amplifier 3 2 Hz–3 2 kHz The calibration of all was performed biologically to correspond ISO threshold values and is expressed as d bels hearing level (dB HL)

Identification of the responses

The response obtained using trans tympa ECochG is a complex mixture of several ferent bio-electric potentials Each of th separate components was identified using following techniques

The action potential (AP) and the summ ing potential (SP) are d c responses whilst cochlear microphonic (CM) is an a c po tial To isolate the a c and d c responses the number of stimuli are delivered in phase (initially up going) and half the st are delivered in the exact opposite phase tially down going), and the responses to are addressed by multiplexing to sepa memory stores Store A contains the AP and CM to one phase of stimulation and B contains the AP, SP and CM to the opp phase of stimulation When the stores A a are added (A+B), the AP and SP are displ with the CM suppressed When using higher audiometric frequencies (above 1 k this phase reversal makes little differen the averaged AP waveform but at lower quencies it leads to distortion as each i vidual nerve impulse is initiated by the ward (towards scala media) movem the basilar membrane (Elberling & Sal 1971)

The CM may be isolated if the stores A B are subtracted (A–B) This CM is not bred as it is the averaged CM to one phas stimulation added to the inverted aver CM to the other phase of stimulation It not reflect the actual CM to one phas stimulation alone and so CM distortion present could be overlooked Neverthe

method does lead to the suppression of of the AP waveforms, especially at the frequencies and to complete suppres of the SP

SP may be identified due to its lack of ilibration The d c responses are first d by using alternating stimuli of oppos ies (suppressing the CM) at a rate of imuli per second and the results addressed ne memory store A, under these circum ces the AP is displayed at its maximum litude together with the SP Next, the re nses are obtained using the same stimuli rate of 100 per second and they are ad sed to memory store B, this time the AP ws the effects of adaptation (the exact of adaptation depends mostly on the stimulus interval, but using the click nuli described at a rate of 100 per second, AP is reduced to about one third of its true plitude) whilst the SP component is unaf

Thus when the averaged responses in each mory store are subtracted (A–B), the AP is played at about two-thirds of its maximum itude whilst the SP component is sup It is quite simple under these circum to judge the extent to which the SP, or similar potential, is affecting the SP/AP rm However, the AP waveform ob- after subtraction is distorted under conditions since at a stimulus repetition of 100 per second, the latency and width the AP are also affected (Eggermont & oor 1973) so subtraction of the memory leads to some differentiation of the AP

In this series of patients the AP and CM ses were noted throughout each re ng Particular attention was paid to the waveform since this has been shown to widened in cases of Meniere s disorder ibson & Beagley, 1976) This widening has found to be due to an enhanced SP (or like) component It has been recently that in general patients with an en iced SP waveform benefitted more from saccus surgery than those with a more normal waveform (Gibson et al , 1976)

The summating potential

In 1950 Davis, Fernandez McAuliffe and Bekesy independently noted that a tonal stimulus can elicit a d c potential change in the normal cochlea at high stimulus intensities and since then the SP has been the subject of a great deal of research The SP is a multi component response representing the sum of various non linearities occurring within the cochlea The exact sources are still a matter of conjecture Whitfield (1965) showed that a major component of the SP was derived from an asymmetry in the mechanism that produces the cochlear microphonic

The non linearity producing an increased negative SP in endolymphatic hydrops, may be due to displacement of the cochlear parti tion and thus asymmetry in the mechano-elec- tric phenomena associated with hair cell stimulation Durrant & Dallos (1974) have shown that mechanical displacement of the basilar membrane alters the SP recorded with intra cochlear electrodes When the basilar membrane is displaced towards the scala media by raising the pressure in the scala tym pani, the negative SP decreases in amplitude and may even change its polarity Conver sely, when the basilar membrane is displaced towards the scala tympani as would occur in endolymphatic hydrops the negative SP is enhanced Similar results have been obtained after biasing the basilar membrane with low frequency sound and concurrently recording the SP using a transient high frequency stimu lus (Durrant & Dallos 1974) An increase in the endolymphatic potential also accentuates the negative SP (Durrant & Gans, 1975)

Patient selection

Eighteen patients, attending the London Hos pital, were assessed using transtympanic elec trocochleography during glycerol dehydration All patients were felt, clinically, to be suf fering from Meniere's disorder The disease

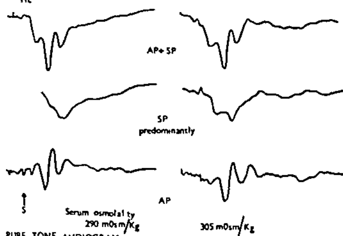
Fig 1 Marked decrease in the tive SP observed 1 hour after t admin strat on of glycerol The shaded area in the pure tone gram represents the increase in threshold (*S* = stimulus onset)

was bi lateral in 2 patients (11 %) Audiometric findings indicated a recruiting hearing loss and the Hallpike Fitzgerald caloric test usually revealed a variable degree of paresis It was evident that the certainty by which the diag nosis had been reached varied considerably Four patients (22 %) had all the classical mani festations of early Meniere s disease including a fluctuating low frequency hearing loss It should be stated however that it is often dif ficult to determine whether the hearing loss is fluctuating The patient may not notice if the variation in hearing is small and regular esti mations of the pure tone threshold may fail to show any convincing change Out of the 18 pa tients, 3 were excluded due to an unreliable electrodochleographic response post caused by poor electrode contact Two ther patients were excluded because change in the plasma omsolality, followi hydration was less than 10 mOsm/kg Th patients were included in this series mean change in plasma osmolality was mOsm/kg with a range of 10–40 mOsm/kg

Table I

13 ears were tested

[illegible]



PURE TONE AUDIOGRAM

SPEECH — NO CHANGE

2·5μV

1ms

Fig 2 A smaller decrease in the negative SP following glycerol dehydration than in Fig 1 The change in the width of the SP is more noticeable than the change in the amplitude

RESULTS

Changes in pure tone threshold and i discrimination following rerol dehydration

ents were regarded as having had a significant change if the pure tone threshold (PTA) roved by 10 dB or greater at two or more iometric frequencies A significant change speech discrimination was taken as an ovement of 10% or more in the maximum mination score

Electrocochleography

me variation in the amplitude of the AP CM occurred during the two-hour test pe in some of the patients This was possibly e to alteration in electrode contact It was therefore felt that under these circumstances conclusions drawn from changes in the amplitude of the AP and SP should be guarded However the width and amplitude of the SP was easily assessed and indeed alteration of the SP was seen to modify the widened AP/SP complex considerably *The negative SP was seen to decrease during glycerol dehydration*

In 4 patients the SP decreased maximally at 1 hour and after 2 hours the negative SP had increased again (see Fig 4)

Table II

13 ears were tested

9 ears definite decrease in negative SP (>0 5 μV)−69 2%
2 ears probable decrease in negative SP (<0 5 μV)=15 4%
2 ears no change in the SP=15 4%

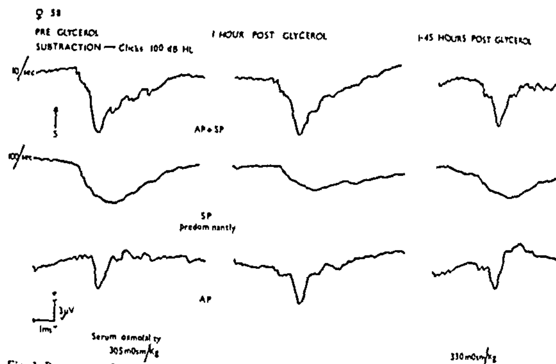


Fig 3 Progressive decrease in the negative SP up to 1 45 hours post *glycerol*

DISCUSSION AND CONCLUSIONS

The striking feature of this study is the marked decrease in the negative SP during glycerol *dehydration* It would seem possible that a decrease in the endolymphatic hydrops produces a movement of the basilar membr wards the scala vestibuli thus decreasi asymmetry in the mechano-electric mena associated with the hair cells A tively the dehydration may decrea

+ THE 9 EARS WHICH SHOWED A DEFINITE DECREASE (>0·5μV) IN THE SP AFTER GLYCEROL DEHYDRATION 4 CHANGED MAXIMALLY AT 1 HOUR AND AFTER TWO HOURS THE SP HAD INCREASED AGAIN

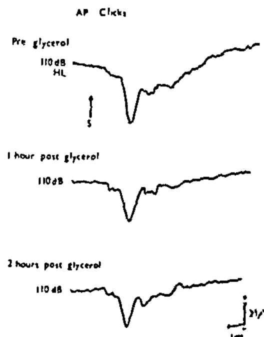


Fig 4

ɔlymphatic potential primarily, leading to creased negative SP *In 4 out of the 9 pa-* ts where the observed decrease in the ne- ve SP took place, the change was maxi- n at 1 hour and thereafter the SP increased ı many patients with Meniere's disorder *e may be no evidence of a fluctuating low* hearing loss and no change in the pure threshold or in speech discrimination fol ıng glycerol dehydration Yet Morrison 5), has shown that, even in this group, *us surgery is often beneficial*

lectrocochleography, however, appears to a more sensitive indicator of changes oc- ring in the cochlea secondary to glycerol ydration than subjective tests

Vine of the 13 patients tested have under- e endolymphatic sac surgery but are only he early postoperative period It would not justified to classify these as successes or ures at this stage and the patients are dergoing prolonged follow-up, since long ural spontaneous remissions may occur

t is to be hoped that an observed decrease the negative SP during glycerol dehydration y prove to be a useful pointer to the prog- sis of *endolymphatic sac surgery*

ZUSAMMENFASSUNG

zehn (13) Patienten mit der Ménièreschen Storung rden bei Anwendung einer Glyzerol Dehydration mit *fe von transtympanalen* Elektrocochleogrammen ge tet Der haufigste Befund war eine Verminderung des ₃ativen Summierungspotentials Es stellte sich dabei raus dass dies ein *feinerer Indikator für Veranderungen* der Schnecke war als Rein Ton Audiometrie und rachdiskrimination Es wird im folgenden der Versuch ternommen eine pathophysiologische Erklarung dieser obachtung anzubieten und die Moglichkeit der Nutz rmachung dieser Verminderung des negativen Sum erungspotentials als ein Hinweis zur Prognose endo- npathischer *Sackchirurgie* wird diskutiert

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Appendix

Patient's initials	Change in serum osmolality (mOsm/kg)	Change in PTA (dB)	Change in speech discrimination (%)	Electrocochleography	
				AP/SP width (ms)	decrease in SP (μV)
S N	15	Nil	Nil	2 9	0 6
T I	10	↑ 10	↑ 20	2 4	0 6
I R	15	↑ 10	Nil	4 0	0 5
D C	12	Nil	Nil	2 9	0 55
A M	15	Nil	Nil	7 8	Nil
M B	18	Nil	↑ 24	0 75	0 4
A M	*40*	*Nil*	*Nil*	*2 5*	*Nil*
P K	15	↑ 10	↑ 10	3 75	0 5
D O	12	↓ 10	Nil	3 2	0 2
E L	25	Nil	Nil	4 0	0 6
D W	25	Nil	Nil	2 9	0 8
C T	10	↑ 30	Nil	5 9	1 0
V N	15	Nil	Nil	2 5	0 5

[illegible]

Decrease in the negative summating potential (SP) refers to the maximum observed decrease in the amplitude from the baseline in micro-volts using the subtraction technique as described in the text

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COCHLEAR FREQUENCY SHARPENING—A NEW SYNTHESIS

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act Recent evidence indicates a substantial dif e in sharpness of tuning between basilar membrane anics and primary neuron responses in mammals paper describes a new qualitative model for a ening mechanism It is suggested that the inner hair are sensitive to d c potential changes in scala media are induced by sound stimuli and that these d c po- ils can suppress neuron activity in a frequency ndent way The model explains sharpening of both of neural tuning curves the shape of the low ency part of the tuning curves and is also compatible other phenomena such as two-tone inhibition and the ts of electrical polarization of the basilar membrane

s paper summarizes data defining the prob- of frequency sharpening in the mammalian hlea and which place profound constraints models of sharpening mechanisms A new othesis, which is particularly suitable for erimental analysis, is presented and inte- ted with these data

Békesy (1960) measured the frequency ectivity of different basilar membrane loca is in various species by determining the ind pressure levels (SPL's) necessary to duce a certain displacement over a range of quencies More recently, similar responses he guinea pig (Johnstone et al , 1970, Wil- & Johnstone, 1975) and squirrel monkey iode & Robles, 1974) have been measured gher frequency locations in the cochlea at much lower SPL's The new data are in ? - with those of Bekésy, but sharpness of tuning is higher at these frequencies Basilar membrane dis- placement for constant SPL on the low fre- quency side of the best frequency falls off initially at less than 5 dB/octave On the high frequency side, displacement falls off at about 130 dB/octave (Wilson & Johnstone, 1975), (Fig 1)

Primary auditory neurone selectivity has been measured in the eighth nerve (Evans, 1972, Geisler et al 1974, Kiang & Moxon, 1975) and spiral ganglion (Robertson & Man ley, 1974) of mammals, including species used for basilar membrane determinations These single neurone response patterns are re markably consistent across species and, in ani- mals in good physiological condition, their fre- quency selectivity is higher than that of the basilar membrane (Evans, 1974, Robertson & Manley, 1974) Even if it is assumed that coch- lear hair cell responses are more related to basilar membrane velocity than to displace- ment, the differences are still large (Fig 1) Typically, low frequency slopes of neurone tuning curves are more than 100 dB/octave near the most sensitive, or characteristic fre- quency (CF) On the high frequency side, slopes are commonly several hundred dB/oc- tave (Fig 1)

A saturating non linearity found on the squirrel monkey basilar membrane (Rhode & Robles, 1974) at higher sound intensities does not appear to be a general phenomenon, as it

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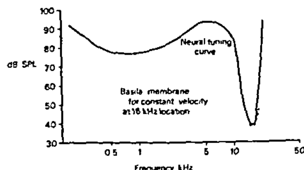


Fig 1 A comparison of the mechanical frequency response of the 16 kHz location in the guinea pig cochlea and the frequency selectivity of a single neurone with CF 16 kHz on the same axes The mechanical response is plotted as the sound pressure level necessary to produce a constant velocity in the 16 kHz location Its position on the intensity scale is arbitrarily adjusted so that the most sensitive response is at the same level as the neural CF threshold Because the most complete tuning curves are from the cat the neural data here are from Kiang & Moxon (1975) Deviations from guinea pig tuning curves are unlikely to be large but may be significant at low frequencies The dB differences between these curves are plotted in Fig 2B

did not appear in the guinea pig (Wilson & Johnstone, 1975)

The second filter

Recent evidence confirms that in normally operating cochleas a fundamental difference in selectivity does exist between the mechanical and neural responses (Evans & Wilson, 1975, Geisler et al , 1974) Some of the models for a "second filter" which creates this sharpening effect, particularly those models requiring complex mechanical transformations in the organ of Corti are difficult to test experimentally While many investigators favour some kind of non-classical inhibitory interaction between fibres innervating inner hair cells (IHC's) and outer hair cells (OHC's) (Evans, 1975, Lynn & Sayers, 1970 Zwislocki 1975), experimental tests have many methodological difficulties Current techniques which aim to 'selectively eliminate OHC's from certain areas of the cochlea (e g ototoxic drugs, Ylikoski et al 1974) are not sufficiently selective (Manley 1975) It is not certain that nerve recordings from IHC only regions (Zwislocki, 1975) originate from normal IHC's, for response changes could be [illegible] to lack of OHC interaction or to IHC [illegible] damage which is hard to detect, or both [illegible]tron microscopy reveals changes in hair [illegible] which look normal with light microscopy ([illegible]koski et al , 1974) Damaged hair cells d[illegible] always disappear (Ryan & Dallos 19[illegible] can continue to be present and pres[illegible] abnormally functioning even months [illegible] damage (Spoendlin, 1976) Disarrangement [illegible] the sensory hairs of IHC's appears only in [illegible] toxic degeneration, the functional signific[illegible] of which is unknown (Ylikoski et al [illegible] Zwislocki (1975) obtained abnormal re[illegible]ings from hair cell regions which looked [illegible]mal in their surface preparations Even [illegible] the whole organ of Corti appears normal [illegible] loss can be seen (Pinheiro et al , 1973) [illegible] fibre activity recorded from ototox[illegible] damaged cochleas is very hard to inte[illegible] None of the various theories of co[illegible] sharpening mechanisms has thus been th[illegible]ject of definitive experimental verific[illegible] Ideally, models should be testable in co[illegible] with an intact complement of hair cell[illegible] with little interference

Constraints on the model

I summarise here the features of recen[illegible] which a model must either try to explain [illegible] compatible with Some of these deta[illegible] based on more solid evidence than othe[illegible] for the present purpose will be treated [illegible]sumptions

Hair cells

1 Intracellular response potentials in [illegible] line (Flock et al , 1973) and basilar [illegible] (Weiss et al , 1974) hair cells are small [illegible]ally less than 3 mV well above threshold [illegible]pared with most other sensory cel[illegible] secretory membranes of some hair ce[illegible] thus very sensitive to potential differen[illegible]

2 Recent findings indicate that [illegible] dominate the production of cochlear [illegible]phonic (CM) and summating potential ([illegible]

proportion to their relative number (Dal-
t al , 1972*a b*)
Stereocilia of IHC's are probably not at-
d to the tectorial membrane and thus will
imulated by fluid velocity in the sub-tec-
l space While IHC microphonic is pro-
onal to basilar membrane velocity, OHC
ophonic is proportional to its displace-
t (Dallos, 1973) OHC stereocilia are firm-
nserted in the tectorial membrane (Ho-
o, 1974, 1976, Kimura, 1966, Kosaka et
1971, Lim, 1972, Ross, 1974) For IHC's,
ion varies, ranging from a type of attach-
t similar to that of OHC's (Iurato, 1967,
, 1974), IHC attachment in some parts of
cochlea of only certain species (Hoshino,
), to no connection at all (Kimura, 1966,
leman et al , 1971)
uring ontogeny, the tectorial membrane
rates more and more from the hair cell
s (Hoshino, 1974) The available evi-
ce, taken together, indicates that the sepa-
on goes further in the IHC area than the
C area In cats, IHC's of the basal turn
ke impressions in the tectorial membrane,
shallow imprints show that even in this re-
n any possible connection is less firm than
t of OHC's (Hoshino, 1976)
n the guinea pig, there are no impressions
m IHC s Even the indistinct imprints in
t of the cat's cochlea may be only remnants
ontogenetic processes
IHC and OHC organelles show con-
cuous differences in size, arrangement and
tribution (Spoendlin, 1975) IHC afferent
synaptic structures appear to conform
re to the typical chemical synapse arrange-
nt than do the afferent synapses of OHC s
unn 1975)

ervation

More than 90% of the afferent innervation
minates on IHC's, apparently with most
s only contacting a single IHC Although
s make up about 80% of the receptor cell
they receive less than 10% of the
innervation (Spoendlin, 1975)

2 There appears to be no anatomical evidence for pathways which could obviously mediate interactions between OHC and IHC afferents Classical lateral inhibition is ruled out by this anatomical evidence and by the fact that the tuning properties of the afferent system do not require time (e g , synaptic delays) to develop (de Boer, 1969, Evans, 1975*b*, Moller, 1972)

3 There are conspicuous metabolic differences between IHC and OHC dendrites (Spoendlin, 1975), e g IHC dendrites are acutely sensitive to anoxia, whereas OHC dendrites are unaffected by brief exposure (Spoendlin, 1973) Similarly, sectioning of the auditory nerve leads to degeneration of the IHC afferents in a few weeks, whereas OHC afferents survive more than a year (Spoendlin, 1973, 1975)

Afferent fibre activity

1 Microelectrode recordings from the eighth nerve (Evans, 1972, Kiang & Moxon, 1975) or spiral ganglion (Robertson & Manley, 1974, Manley & Robertson, 1976) sample a homogeneous population of cells For example, in any one location in the spiral ganglion of an individual guinea pig (fibres of identical CF), the range of thresholds is only 6 7±3 9 dB (Robertson, 1975) Either the adequate stimulus for the outer spiral fibres has not been found (assuming they do not show spontaneous activity) or the primary function of OHC afferents is not the transmission of auditory input to the central nervous system

2 While the crossed olivo-cochlear bundle (COCB, one component of the efferent pathway) appears to have most of its inhibitory terminals on OHC somata (Spoendlin, 1973), its activation affects all primary afferent fibre activity in an inhibitory fashion (Wiederhold & Kiang, 1970)

Pre neural sharpening

One possibility for a sharpening mechanism which has not received much attention is pre neural sharpening via interactions between

hair cells directly through the extracellular space of scala media Earlier model stimula tions (Strelioff, 1973) have demonstrated that shunting of current by nearby hair cells must occur in the cochlea, and such an interaction could affect neural activity under certain con ditions This idea is similar to the notion that hair cells might be affected by small potential changes in the endolymphatic space, produced by the activity of neighbouring hair cells

I believe the features of the recent data summarized above are best accounted for by the following general scheme All afferent fibre ac tivity is recorded from IHC afferents OHC afferents are silent but necessary for OHC maintenance OHC's are specialized as variable channels for current to flow out of scala media in quantities sufficient to affect the resting potential level of scala media (the endocochlear potential or EP) This flow of current occurs, or is increased, during sound stimulation or during COCB activation IHC's are sensitive to both the fluid velocity in the sub tectorial space and to d c electrical changes induced by OHC activity (negative summating potentials, or −SP are of most interest here, reaching values of 3–5 mV in scala media in the normal recording locations) (Dallos et al , 1972*b* onrubia & Ward, 1969*a*) This −SP sup esses sound evoked activity in IHC's Whether or not the output of an IHC synapse changes is assumed to depend both on the mechanical stimulus and the size of the −SP (Manley, 1976)

It is not unreasonable to suggest that the hair cells producing the neural output (the IHC's) might be sensitive to small changes in their electrical environment as vertebrate hair cells (neuromasts) and teleost electroreceptors have a common origin in mechanoreceptive lateral line cells of fish (Bennett 1970) Both mechanical and electrical sensitivity of neuromast receptors (Suga 1967) are considerably enhanced by high potassium ion concentra tions over the hair cell (Hashimoto & Katsuki 1972), as in the endolymph of the cochlea *Even without such high potassium levels neuromast derivatives can have h* [illegible] tivity Tonic electroreceptors, for [illegible] are sensitive to external d c potential [illegible] much smaller than the typical recep [illegible] tials of receptor cells A swing of [illegible] voltage from −1 mV to +1 mV is suffic [illegible] take tonic electroreceptor afferent f [illegible] Gymnotus from 0 to 300 impulses per se [illegible] their entire dynamic range (Bennett 197 [illegible]

To illustrate how such an electrical se[illegible] tivity on the part of IHC's could sha[illegible] neural tuning curves, consider an experi[illegible] to determine the threshold response [illegible] curve for a neurone in the guinea pig [illegible] nerve most sensitive to a tone of freq[illegible] 16 kHz (the CF) At the extremely low S[illegible] necessary for a response at the CF, the −S[illegible] this CF location would be extremely s[illegible] (Honrubia & Ward, 1969*a*) This tiny [illegible] would have little or no suppressive eff[illegible] the IHC and thus the low mechanical thre[illegible] of the cell is not affected When stimulus [illegible] quency is dropped an octave to 8 kHz SPL would have to be raised 3–4 dB (W[illegible] 1974) to compensate for the smaller displ[illegible] ment in the CF location to this new tone H[illegible] ever if the IHC's are velocity sensitive SPL would have to be raised an add [illegible] dB to produce the same velocity in th[illegible] location (I assume that the velocity nece[illegible] to induce a threshold conductivity increa[illegible] the IHC is achieved at CF at the same SP[illegible] the displacement necessary to induce a th[illegible] old conductivity change in OHC's)

The new travelling wave envelope prod[illegible] by the 8 kHz tone peaks about 2 5 mm [illegible] ward of the CF location (Wilson 197[illegible] because of the 9–10 dB increase in SPL s[illegible] lates a much larger area of cells At the [illegible] location, while the velocity of the basilar m[illegible] brane again matches that of the CF thres[illegible] the OHC's are being stimulated at a level [illegible] above that of the CF frequency and thres[illegible] They therefore undergo a larger resist[illegible] change and the −SP produced by all the [illegible] cells is much larger The model supposes *the final −SP in the CF location produce*

3 kHz tone is large enough to suppress the response to the mechanical stimulus The must, therefore, be raised in intensity Of se a higher SPL will produce not only ter mechanical stimulation but also a lar- SP There is thus an antagonism between rising mechanical stimulus and the increas −SP The point at which the suppression omes insufficient to prevent increased aptic output is the new threshold The e reasoning would apply to frequencies of ulation higher than the CF Here, although hing velocity in the CF region is attained ore matching displacement, the slope of the ug curve of the basilar membrane is so p that OHC's in the new peak location are iving very large inputs and therefore pro- ing a large −SP in the nearby CF region

he lateral spread of suppression is limited the space constant of scala media (1 5 to m, Strelioff, 1973, or about 10 dB/octave), on the low frequency side of the tuning ve the suppressive effect tails off when the k of the travelling wave is very distant from CF location On the high side of the tuning ve, the peak locations never become too away to be effective The model thus ac- nts for sharper neural tuning curves on h the high and the low frequency sides of tuning curves and for the progressive re tion of the effect at low frequencies (the so led 'tail of the tuning curve, Kiang & xon 1975) When the animal is made oxic, or otherwise has its auditory sensi- ty artificially depressed, the tuning curve uld on this hypothesis, become broader obertson & Manley, 1974) A possible addi nal consequence of this antagonism might a broadening of the dynamic range of IHC tput Under hypoxic conditions also, dy nic ranges at CF are reduced to half their ginal value (Evans, 1975, Robertson, 1975)

If the above scheme is valid as a working pothesis there should be a good correlation tween the size of the −SP in the CF location neurone threshold for any given frequency d the discrepancy at that frequency between the mechanical velocity curve for the CF loca tion and the neural tuning curve From my own (unpublished) data and from published accounts (Dallos et al , 1972*b*, Honrubia & Ward, 1969*a* Manley & Johnstone, 1974) a good estimate can be made of the −SP present in, for example, a 16 kHz CF location at the frequencies and intensities which form a threshold curve for a neuron in that location (Fig 2) By normalizing the frequencies I com pare directly the −SP at a lower frequency location, such as for a CF 2 8 kHz unit from Evans (1972) The resemblance between the two functions is good the largest −SP s being generated near normalised frequency 0 6, or less than an octave below the CF This cor responds well to the frequency at which the low frequency slope of the tuning curve of a neurone becomes less steep Below this fre quency, the discrepancy between mechanical and neural curves also decreases (Fig 2)

Two tone suppression

The idea that −SP present at the CF location but mainly produced in another location sup- presses activity in the CF location is relevant to the phenomenon of two-tone suppression or inhibition It is well known (Arthur et al , 1971, Sachs, 1969, Sachs & Kiang, 1968) that when a tone at the CF and a little above threshold stimulates a neurone the response can be suppressed by a tone of higher or lower frequency which when presented alone, is not in most cases loud enough to stimulate the cell According to my model, the −SP present at the CF location and generated by the tra- velling wave of the second tone could suppress the response Quantitative interpretation is complicated by the fact that both tones pro- duce some mechanical input to the CF location and both produce some −SP It is noteworthy that the sharpness of the tuning of the in hibitory areas and of the single tone excitatory areas increases with increasing CF (Sachs & Kiang, 1968), suggesting that the mechanisms of normal sharpening and two-tone suppres sion share common features Kiang & Moxon

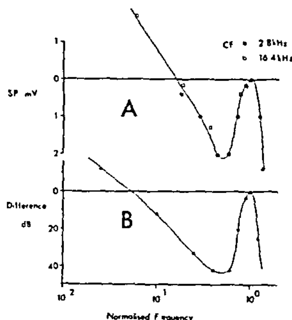


Fig 2 (A) Size and polarity of SP produced in scala media by the frequencies and intensities normally used to obtain threshold responses from two neurones of different CF. The SP data for the tuning curve of the neurone with CF 16 4 kHz are for the appropriate location in scala media of the basal turn of the cochlea and for the neurone with CF 2 8 kHz are from scala media records from the second turn. The frequency of the CF's has been normalized to 10^0 and other frequencies are represented as fractional values of this. The curve is an eye fit to both sets of data points. (B) Difference between mechanical and neural curves of Fig 1

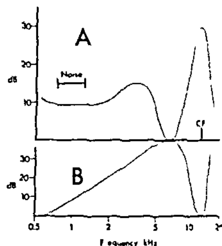


Fig 3 (A) Diagram of the inhibitory effect on different portions of the tuning curve of a neurone of CF 1… of a 500 Hz wide band of noise centred at 1 kHz as calculated from data of Kiang & Moxon (1975). The … intensity was about 15 dB higher than that which … alone just evoke a response from the unit … fects for noise bands both more and less … similar shapes. This noise band produces a 30 dB … threshold to CF tones but has no effect on … tones near 6–7 kHz (B) Inhibitory function … vironment of the IHC's in the CF location … from (A) that the noise band has no effect on the … *curve at a frequency where the inhibitory f*… cates that a large amount of suppression already … The maximum of this inhibitory function is … placed at 40 dB to compare with Fig 2B

1975) show the suppressive effect of low frequency noise bands on high frequency tuning curves. These noise bands alone just produced activity in the unit for the lowest intensities used but suppressed parts of the single tone tuning curve. Fig 3 presents a graph of this frequency dependent suppression for a unit of CF near 13 kHz as calculated from their data. The maximum effect of the noise is near the CF, where my hypothesis predicts that there is no pre existing suppression. There is no effect about an octave below the CF where there is already a large existing suppression. These frequency-dependent effects are therefore highly compatible with this model.

The response of a neurone at CF and off CF are therefore seen as the result of different degrees of interactive forces. This is in keeping with the finding that the tips of tuning curves behave differently than the tails … toxic drugs, high intensity sounds, COCB stimulation and low frequency masking … tuning curves mostly at the tips (Kiang & Moxon 1975). In essence the tuning … represents a response already partially … by suppression, and the tails are … much less susceptible to interference

Efferent suppression

An additional value of this model is … understand the frequency-dependent … COCB stimulation. According to … (1974*a* *b*) the COCB could act via its …

ncreasing the OHC conductance, shunting nt away from IHC s Thus, the IHC shold would be increased and the rate tion moved to higher SPL's In my model, COCB makes use of an existing suppres- mechanism for affecting IHC output by lifying its electrical environment The imeters normally used (Sachs & Kiang,) to stimulate the COCB produce a slow in the EP of 3–4 mV My model predicts this change would be suppressive near the but at off CF frequencies the effect would ibine with pre existing suppression in a iplex way, as SP itself is affected by COCB ulation Thus the effect should be maximal r the CF, as is the case (Wiederhold & ig 1970) Any explanation of COCB ef s is complicated by the fact that a propor- of COCB fibres in some species form dendritic synapses on IHC radial afferents oendlin, 1975)

ificial polarization of the cochlea

ctrical polarization of the cochlea changes magnitude and even the sign of the EP and cts the other dependent potentials (Hon ia & Ward, 1969*b*) It also affects the ac- ty of eighth nerve fibres (Konishi et al , 0 Teas et al , 1970) Unfortunately, in the ter case, the magnitude of the EP and SP fts produced by the polarizations were not asured According to Honrubia & Ward 969*b*) however, the current crossing the silar membrane under these conditions is s than one tenth of the total, so that the tential changes were probably not large It is ly possible at this stage to see if the data is alitatively compatible with my hypothesis In the first place if the imposed current flow poses the normal outflow of ions from scala edia through the hair cells, polarization ould be expected to suppress the responses tonal stimulation, and such is the case in % of the fibres I will discuss the other 10% low This is only true, if the tone used is at near the CF At other frequencies, such cur nt *increases* tone evoked discharge rates (Teas et al , 1970) This anomalous discharge increase is explained by my model by supposing that the dominant effect of the current for tones off the CF is to oppose the outflow from scala media of a current which is sup pressing the fibre, i e , to 'disinhibit' the fibre There being no significant suppressive cur rent from tones at the CF near threshold, the current then suppresses the unit s activity With regard to the 10% of the units which in crease their discharge at the CF, I suggest that this is due to an inaccurate estimate of the CF Teas et al (1970) state that due to the limita tions of their equipment they did not measure accurate tuning curves

Comparative aspects

Given that all neuromast derivatives have some degree of both mechanical and electrical sensitivity, it is reasonable to suppose that the generalities of the scheme outlined above apply also to the ears of non mammalian vertebrates Granted that mammals have greater specialization of both hair cell populations and innervation patterns but the basic idea would apply to any spatial array of receptor cells which are influenced by potential changes or current flow changes induced by their neighbours Thus some of the factors in this model might also be involved in the sharp tuning found in non mammal primary fibres, and the two-tone inhibitory phenomenon in birds (Sachs et al 1974)

Experimental tests

This hypothesis is offered in the hope that it will stimulate both new experiments and formal quantitative analysis taking more factors into detailed consideration At the moment it seems that cochlear frequency sharpening can best be explained by a combination of some thing like the present hypothesis and some sort of differential coupling of the IHC cilia to basi lar membrane motion at different frequencies (Duifhuis, 1976 Zwicker, 1976) Some rela tively straightforward tests of the present hypothesis looking at the effects of modifying

the electrical environment of the hair cells on tuning properties, would bring us closer to the solution of this interesting and important problem Auditory masking experiments which have demonstrated longitudinal cochlear interactions probably do occur (Ryan & Dallos, 1975*b*) might also be re examined in relation to the present hypothesis

Finally, it should be noted that analogous cases of interaction via electrical lateral suppression have recently been described (I attempted in this paper to retain the term inhibition' for events mediated by special intercellular junctions, and suppression for a phenomenologically similar event mediated differently) Mutual suppression of different colour receptors of the locust eye has been shown (Shaw, 1975) Due to the pattern of extracellular resistances ions from strongly stimulated receptors flow out through weakly responding ones (with different colour sensitivity) and suppress their activity Similar contrast enhancing mechanisms operate for polarized light sensitivity in the crayfish eye (Muller, 1973) As Shaw points out, this system can produce selective and mutual suppression without needing complex genetic programmes for the inhibitory wiring of neural af ... This kind of lateral electrical suppression is a phenomenon whose importance in ... arrays is only now beginning to gain recognition

NOTE ADDED IN PROOF

Two important papers have appeared since the completion of this manuscript Russell and Sellick (1977*a*) demonstrated that the receptor potentials of the inner hair cells of guinea pigs are much larger than expected (up to 15 mV) and that these receptor potentials are just as sharply tuned as the nerve fibre responses Although the latter finding is predicted by my model measurements of impendance changes in hair cells during tonal stimulation (Russell and Sellick 1977*b*) place important restrictions on the applicability of the mod[illegible] described here

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I thank R R Capranica R Chase E. F Evans R Funnell R Levine J P Wilson and J Winslow for [illegible] discussions R Funnell and J Winslow for computer programming and L. Pawson for technical ass[illegible]

ZUSAMMENFASSUNG

Neue Ergebnisse weisen darauf hin daß es [illegible] lichen Unterschied in der Frequenzselektivität [illegible] der Basilarmembran und den primären Neuronen [illegible] Saugetieren gibt Diese Arbeit beschreibt ein [illegible] tatives Modell das die Verbesserung der Frequenzselektivitat erklärt Hierbei wird angenommen daß die [illegible] Haarzellen durch reizbedingte Veranderungen des [illegible] strompotentials der Scala media beeinflußt werden [illegible] daß diese Potentialanderungen die Aktivität der [illegible] Haarzellen frequenzabhängig unterdrücken [illegible] Modell erklärt wie im Vergleich zur Basilarmembran beide Seiten der Schwellenkurven der primären Neurone steiler werden und berucksichtigt auch die [illegible] niederfrequenten Anteils der Kurve Gleichzeitig [illegible] kompatibel mit der Zweitonhemmung und dem [illegible] von elektrischer Polarisation an der Basilarmembran

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RELATION BETWEEN THE WAVEFORM OF THE COCHLEAR WHOLE NERVE ACTION POTENTIAL AND ITS INTENSITY FUNCTION

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tract The whole nerve action potential (AP) was re ded by intracochlear electrodes in the guinea pig As vell known in normal conditions the AP elicited by e bursts displays negative and positive deflections In ivation of the central end of the nerve at the internal litory meatus (IAM) by introduction of a few drops of l solution or by mechanical pressure, produces a inge in the wave shape of the AP which is transformed o a single negative deflection The relation of the am tude of the monophasic AP to the intensity of the tone rst is monotonic in contrast to the classical two-slope nation observed in normal conditions These results interpreted by the disappearance of a positive com nent of the response produced at the IAM The con lution of the monophasic unit AP would explain the notonic intensity function

avis et al (1952) gave an interpretation of the hole nerve action potential (AP) recorded by ectrodes in contact or penetrating inside the inea pig cochlea They indicated that the ource of the AP was localized at the level of e internal auditory meatus where the tightly athered fibres pass through the bone They ointed out that because the nerve traverses ı insulating bony tube, two physiological lectrodes are constituted by the two openings t the tube These anatomically determined lectrodes represent the effective recording ites when the outputs from wires placed in cala tympani and scala vestibuli are summed nd expressed against a remote grounding oint on the animal Since such physiological lectrodes should produce diphasic neural responses (AP), Teas et al (1962) presented a model of the AP to explain how diphasic neural elements of the population of fibres could combine in amplitude and phase as a travelling wave excites the elements successively, to produce the classical N_1 and N_2 deflections The accuracy of this model was strongly supported by Legouix & Pierson (1974) who found that inactivation of the internal end of the nerve by KCl solution made the AP recorded by cochlear electrodes monophasic They also observed that the intensity function of the AP was modified when the waveform of the AP was altered These results were in agreement with the model of Teas et al

This paper reports a series of experiments on the guinea pig which were devised to show that the contribution of two sources of AP at both ends of the meatus produces positive and negative waves which combine to produce various resultant waveforms They show also that some of the classical characteristics of whole nerve intensity functions are altered by blocking transmission of nerve impulses at the internal auditory meatus (IAM)

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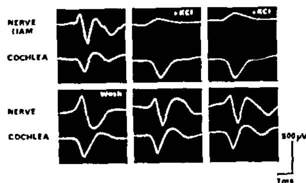


Fig 1 Responses recorded from the aud tory nerve (AP) and internal aud tory meatus (IAM) in the guinea pig to a 7 kHz tone pip with rise time of 1 0 msec The upper left panel shows control waveforms The upper m ddle panel shows the response waveforms after application of KCl to the IAM region Only a small positive deflection remains at IAM probably due to voltage pick up from the cochlear side of the IAM The (cochlear) AP has lost the posit ve peak and is broader than for the control response Upon washing with saline the response beg ns to recover as shown in the lower panels

METHOD

The APs were recorded from guinea pigs (350–400 g) anesthetized with ethylurethane In some cases the animal was curarized and artificially ventilated The recording electrodes were steel or copper wires 100 μm diameter These electrodes were introduced into scala ani and scala vestibuli in the basal turn d sometimes in the third turn according to the classical differential technique Another electrode was usually introduced in the prox imity of the nerve trunk at the internal audi tory meatus (IAM) after opening the skull and removing part of the cerebellum

To inactivate the central end of the nerve a technique similar to that used by Legouix & Pierson (1974) was applied i e a few drops of a KCl solution was introduced (0 1 N) in the vicinity of the nerve This procedure resulted in a decrease in the response under the IAM electrode This effect was reversible and the IAM response recovered in a few minutes The procedure could be repeated safely sever al times In other cases inactivation of the nerve at the IAM was produced by mechanical pressure exerted by a tiny cotton ball intro-duced near the nerve trunk In this ca the effects could be very gradual b re versibility was sometimes incomplet

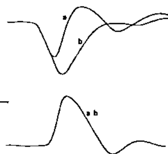
In most experiments the animal was ed with an ear bar on one side and a s into the external auditory meatus on th Clicks or tone bursts were provided by t speaker located 5 cm from the open of speculum The responses were d spa an oscilloscope and photographed In man stances the APs were averaged to impr S/N ratio A total of 40 guinea pigs were in these experiments

Contribution of the IAM Source to the AP Recorded by Differe d Electrodes in the First Turn

To evaluate the contribution of the I source to the AP recorded by the d fer electrodes (their sum) in the first turn th was observed before and after inactiva the IAM source by the KCl solution

The electrode located in the vicinity of internal auditory meatus recorded re sponses with different wave shapes acc to the position of the electrode These tions included changes of polarity and w form which were obviously related to a ume conductor effect In order to facil experimental comparisons several pos of the IAM electrode were successively until a criterion waveform was obtained criterion IAM waveform was obtained the electrode was near the internal me showing a negative peak delayed by 0 25 msec compared with the AP The tive peak was preceded by a slight p voltage coincident with the onset of th negative peak The delay in the negative probably corresponded to the conducti to the electrode site at the IAM (Fig 1)

Most of the characteristics of the ce recorded neural response were similar t of the cochlear first turn i e the AP U the centrally recorded response showed negative peaks the second being so greater than the first and separated by



The effect of KCl introduced at the nternal audi
neatus upon the whole nerve AP response recorded
basal turn different al electrodes Curve *a* control
b post KCl appl cation curve *a–b* d fference be
curve *a* and curve *b* Curve *a* *b* represents the ef
of an active IAM upon the AP response recorded
the basal turn

ec At some electrode locations the peaks ned to correspond to N_1 and N_2 in the llear AP at other positions of the elec es the wave shape could be quite different s similarity between the AP (cochlear) the IAM response is not true for all elec e locations Owing to the latency this re nse could be attributed to first order neu "s However in some records several late ections were strong and probably origi d in secondary or tertiary neurones

fter introduction of the KCl solution at the I the response showed a rapid decrease in plitude and a change in polarity and shape ually a small positive deflection remained ich could be attributed to a passive trans sion from the cochlea At the same time the (recorded by the electrodes in the cochlea) nged its shape and became monophasic as positive deflection between N_1 and N_2 dis peared in such a way that the AP displayed single wave of large amplitude Fig 1 il trates the alterations in the waveforms from cochlea and the IAM Similar effects could obtained with mechanical pressure pro- ced with a probe in the region of the IAM wever the effect of KCl was more progres e and easier to control After rinsing with nger a complete recovery of the initial re onses was observed

In some instances the KCl solution appar ently penetrated inside the cochlea since CM—as well as the cochlear AP—were re versibly depressed When this cochlear de pression occurred the experiment was re jected

The exact correspondence between the dis appearance of the potential recorded at the IAM and the change in the AP suggests that the positive deflection between N_1 and N_2 of the cochlear AP was generated at the IAM Fig 2 shows for a specific pair of waveforms the effect of KCl applied to the IAM The waveform labelled *a* is the AP before applica tion of KCl that labelled *b* the waveform of the AP after application of KCl The stimulus was at the same intensity for both waveforms Waveform *b* follows the onset of *a* but reaches a larger amplitude just before *a* returns to the baseline and continues to its positive peak Both waveforms then reach the baseline sim ultaneously *a* from a positive voltage *b* from its negative voltage The difference between the two waveforms is shown as a third wave

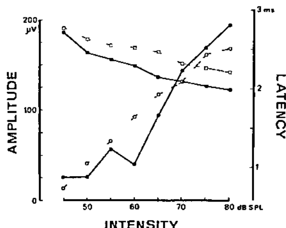


Fig 3 Ampl tude and latency of AP responses before and after appl cat on of pressure-block at the internal aud tory meatus of the guinea p g Stimulus 8 kHz tone bursts rise time is 1 0 ms Pressure was produced by a small cotton ball placed at the IAM The ampl tude of the N response s greater for pressure block than for the control responses The alteration in slope (55–60 dB) for control responses is eliminated for the responses du pressure block

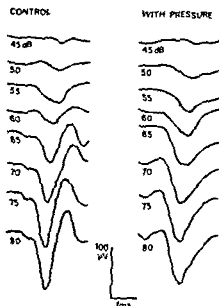


Fig 4 Response waveforms from which measurements shown in Fig 3 were taken

form (a–b) The difference in waveform represents the contribution of the intact IAM region, which was eliminated by the application of KCl

With low frequency tone bursts or low pass clicks, the AP shows a smaller positive deflection but the effect of KCl on the IAM is similar and the N_1 and N_2 are fused together into a single wide negative deflection of large amplitude The increase in amplitude of the AP is sometimes not very obvious because there is apparently a diffusion of KCl towards the cochlea In these cases, however it is possible during the recovery to observe a transitory state where the AP shows an amplitude remarkably increased

Relation between the Shape of the AP and Its Intensity Function

The increase in amplitude which follows the disappearance of the positive deflection suggests that some cancellation of the negative peak has been removed when IAM is blocked This interpretation is consistent with the model of Teas et al which describes the convolution of the neural elements according to their shape and synchrony Because a change in shape of the neural elements may modify the resultant, i e the response waveform and its magnitude, we compared the variation in size of the responses as a function of intensity when recorded by the electrode located at the IAM and in the cochlea (a) in normal conditions and (b) after inactivation of IAM by KCl

The intensity function of the N_1 is well specified for the classical recording situation, i e round window in the cat or basal turn differential electrodes in the guinea pig where the amplitude is measured peak to peak In this series of experiments we measured peak-to-peak and also baseline-to-peak

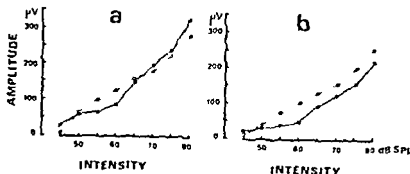


Fig 5 Intensity functions measured as peak to peak (N_1 to positive notch) and baseline-to-peak (N_1) (guinea pig) Stimulus 8 kHz tone burst rise time 1 ms ○—○ post KCl application to internal auditory meatus ●—● pre KCl control responses The two slopes of the AP intensity function are apparent in the control responses The slopes of the post KCl responses do not show the characteristic in the 55–60 dB range of intensities

sually displays two branches, one with ope for intensities below 60 dB and one steeper slope at intensity levels above At the junction between the two es a dip is often observed This shape en considered by many authors as re two groups of receptors activated re- ely by low and high intensities Above the curve is strongly non linear and a ion is observed, followed by a decrease litude

inactivation of the central end of the the intensity function of the cochlear nodified

3 shows plots of amplitudes and la of baseline to peak (negative) of the AP se waveforms displayed in Fig 4 Over f the dynamic range the response ob- while pressure was applied to the IAM ger than the control response The time ch the peak occurred was also later for essure block condition The change in for the pre-pressure block amplitude between 55 and 60 dB is absent in the se for the pressure-block condition the pressure block response shows a lly increasing function without the sepa into the two segments, commonly ob l in the intensity function

5 shows plots of intensity function for sponses to 8 kHz tone pips before and KCl application to the IAM The curves e left are for peak to peak measures, on the right, for the baseline to peak ires

en though the peak-to-peak measure s the observations (the post KCl re e has little or no positive peak), the ge in slope for the pre KCl response in the dB region is lost in the post KCl growth sponse amplitude The baseline to-peak ures show a clear and consistent increase plitude of the AP response following KCl cation

rrelatively, with the changes in the ampli- intensity function, the latency intensity tion is modified In normal conditions, as it is classical, the curves representing the la- tency show two branches coincident with the two branches of intensity function The first branch representing the latency at low inten- sities shows a steep slope, the second branch for higher intensities shows a lower slope After KCl the latency shows a monotonic vari- ation with intensity These coincident changes in amplitude and latency argue that the ampli tude is related to the time distribution or to the duration of the unit potentials

DISCUSSION AND CONCLUSIONS

The changes in the shape of the whole nerve AP have been observed by various authors who attempted to interrupt the auditory path- way at the level of the nerve trunk (Fisch & Ruben, 1962, Kupperman, 1972, Daigneault, 1974) The changes observed were usually in- terpreted as being the result of the removal of the influence of efferent fibres The present results do not contradict this interpretation, but in our opinion it is more likely that the ef- ferent action would change the overall ampli- tude rather than the wave shape Comparison of the cochlear AP with the AP from the IAM after action of KCl strongly supports the inter- pretation that the IAM source participates in determining the waveform of cochlear AP

The increase in size of the cochlear AP after the action of KCl could be related to the sup- pression of a tonic influence of efferent fibres However the interruption of the olivo-coch- lear pathway at other locations, in our experi- ments, did not produce a similar effect It seems more likely that the principal action of KCl was to depolarize the nerve fibres at the IAM, thus blocking the conduction of nerve impulses, and sometimes also to modify the conductivity around the nerve trunk While the change in conductivity could account for the increase in size the saline wash restored the control waveforms Conductivity should be similar for both the solutions The increase in size of the AP may result from the removal of the cancellation of negative deflectio

Ac

the addition of positive voltage, as can be deduced from the model of Teas et al

In subtracting the AP waveform recorded before KCl from the waveform obtained after suppression of the IAM response, the contribution of the IAM to the cochlear AP can be estimated It appears as a positive deflection with a latency 0 3 ms longer than the cochlear AP It is interesting to consider that after KCl the IAM electrode records also symmetrically a positive potential that we assume to be generated at the peripheral end of the meatus

After the KCl action, the shape of AP becomes monophasic and N_2 is fused with N_1 This alteration in waveform seems to result from the disappearance of the positive deflection which divides the response into two parts (N_1 and N_2) Several interpretations have been presented to explain the origin of N_2 It has been suggested that N_2 represents the response of second order neurones Tasaki (1954) asserted that it could be due to a second firing of auditory nerve fibres The disappearance of N_2 after KCl action suggests that it is more likely to be due to the removal of the positive deflection

The intensity function of the whole nerve AP has been studied by many authors The curve which corresponds to it shows two branches which have been attributed to the stimulation of two different sets of receptors (Eggermont 1976) In some instances at the junction of the two curves there is a dip, the origin of which is difficult to explain Recently it was proposed that the two branches were related to the shape of the turning curve of single fibres (Evans 1975)

At low stimulus intensities tuning curves of auditory nerve fibres are very narrow and the AP response probably represents the activation of few nerve fibres from a narrow region along the cochlear partition Thus the lower branch of the curve for the AP shows a small rise with intensity and the function has a low slope At higher intensities the tuning curves are much wider As intensity increases the stimulus produces excitation along a region of the cochlear partition and a g number of fibres produce impulses The breadth of tuning curves for fibres w gressively higher characteristic freq can account for the high intensity bra the intensity function The rapid incre amplitude, i e the steep slope, is d a tivation of fibres with high CFs (Kiang 1977) The present results show ha that the break in the curve disappears attenuated when the AP becomes mono after action of KCl This alteration in intensity function is clearly related to response waveform Thus the inten tion for the whole nerve response is also mined by the specific electro-anatomica tures of the recording procedure in par by the constraint resulting from the d waveform

The low intensity segment of the cl whole nerve intensity function represen pulses from a group of nerve fibres wi acteristic frequencies within a narrow around the stimulus frequency Tone bu 4 kHz or above, with rise times of l are optimal stimuli for eliciting the res At the lowest stimulus intensity there difference between the usual proced phasic response) and the response f IAM block As stimulus intensity is inc the difference between pre and pos block in the baseline to-peak measu creases The data suggest that the p voltage erodes the initial negative goin of neural elements at all but the lowest ulus intensities, and the classical N_1 res can underestimate the response mag more than one half at the strongest s values Estimates of the number of ne ments on the basis of area encompassed in the classical N_1 diphasic response a course, quite inadequate because the form is a complex resultant of the b phasic form of neural activity recorded cochlea

This analysis of the whole nerve A

ise waveform shows that the response it- ı ides no basis for the inference that are two populations of nerve fibres that ‚ selectively to the low and high in v segments of the classical diphasic N_1– "guration Under IAM block, when the aveform is monophasic, the whole- intensity function shows a uniform ı with saturation at high stimulus in ıes

similarity in the form of the classical AP intensity function among species to the similarity of the electro anatomi ints imposed by the configuration ditory nerve and internal auditory mea Differences among species will be in the between cochlear and IAM sources, and ' of excitation toward regions of high ı frequency with increases of in- These two factors may alter the spe- features of response waveforms from human cochlea (electrocochleography) cat, but not the general features

ZUSAMMENFASSUNG

Aktionspotential (AP) wurde mittels Intracochlear roden beim Meerschweinchen gemessen Wie schon ıot ergibt das durch Tonpulse ausgelöste AP unter alen Bedingungen positive und negative Ampli Die Inaktivierung des Nervenendes im inneren us (IAM) durch Aufbringen von einigen Tropfen Losung oder durch mechanischen Druck bewirkt

slope variation welche unter normalen Bedin ıg beobachtet wird Die Ergebnisse werden durch leiben der positiven Anteile der Antwort welche im IAM produziert werden erklart Die Existenz eines einphasigen unitaren APs wurde auch den monotonen Verlauf der Intensitatsfunktion erklaren

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CLINICAL FINDINGS AND DIAGNOSTIC PROBLEMS IN SENSORINEURAL LOW FREQUENCY HEARING LOSS

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Abstract The otological and audiological findings in 39 patients with sensorineural low frequency hearing loss are reported Th s type of perceptive hearing loss is dif f cult to distinguish from true conductive hearing losses due to the air conduction audiogram shape and the in val dity of bone conduction determinations showing a *false* air–bone gap This may lead to surgical treatment of a perceptive hearing loss as reported in the four case histories By various audiological tests contradictory in formation may be obtained In our material Bing s test and absent acoustic reflexes indicated a conductive dis order in 25% of the ears The final differentiation may require cochleography The hearing loss may be diag nosed as Meniere s disease In our material only 17% compla ned of tinnitus and no patients had vert go Con sequently we find sensorineural low frequency hearing loss to differ from Meniere s disease Our material com prises d fferent etiological types of perceptive low fre quency hearing loss One type was inherited as an autoso mal dominant tra t another type due to cochlear mal *formation* probably also inherited and a third group show ing diverse audiological results When the diagnosis is established the patients may be treated successfully by specially constructed hearing aids

Hearing loss may be classified as mainly con ductive or sensorineural Clinically, it may be difficult to distinguish between these compo- nents due to misleading bone conduction determinations (Lierle & Reger 1946) It is of great clinical importance to differentiate be tween these two main types as a conductive hearing loss may be treated surgically Surgi cal treatment of a perceptive hearing loss may fail to improve the hearing or even worse result in further impairment of the hearing ability Also, from a psychological point of view the patient suffers when the hope of i proved hearing is not fulfilled and it may come difficult to motivate such patients wear a hearing aid, postoperatively

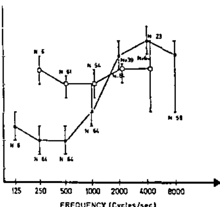
Conductive hearing loss is attributable variety of pathological conditions in the o ear canal or in the middle ear Congenital formation, post traumatic disorder acute chronic infection or sequelae of infecti

malleus syndrome and otosclerosis (Goo 1966, Elbrond, 1970)

Conductive hearing loss is normally cha terized by an air conduction audiogram s which shows a more pronounced hearing pairment in the low frequency area and a creasing loss towards the treble This a metric curve has been named the stiff tilt (Carhart, 1962) The bone condu thresholds are within normal range b some disorders the bone conduction th olds are measured too low, simulating a ceptive hearing impairment (McConne Carhart, 1952, Tonndorf, 1971)

Sensorineural hearing loss is caused pathology in the cochlea the cochlear n or central auditory pathways It is ch terized by progression heredity and have associated abnormality with other c systems (Konigsmark, 1969, 1971*a* 1971

The common type of sensorineural he



1 The median air and bone conduction thresholds ears examined

is characterized by hearing impairment, st pronounced in the high frequency area, a sloping audiogram A special type of sen- ineural hearing loss is seen in the low fre- ncy area, defined as a hearing loss most nounced for the frequency range below Hz and exceeding 20 dB or more The hear- may be better for 125 Hz than for other frequencies (Konigsmark et al , 1971; avendeel & Plomp, 1960, Lundborg, 1955, nce & Sweeney, 1975)

To elucidate the diagnostic problems, we ll report the otological and audiological find- s in the patients with this type of hearing s and evaluate the diagnostic procedures ailable

METHOD AND MATERIAL

r this study, the patients were selected con- -ly according to the otoscopy and the conduction audiogram shape showing hear- loss most pronounced for the frequency - below 2 kHz and exceeding 20 dB or more The air conduction thresholds were obtained with Telephonics/TDH 39 earphones with MX 41/AR cushions, meeting the specifications given in recommendation IEC/177–1965 and calibrated according to recommendation ISO/R 398–1964/Add 1–1970 The bone conduction thresholds were obtained with Oticon A-43 bone conductors applied to the mastoid process, meeting the specifications given in recommendation IEC/177–1965/App A and calibrated on a Brüel & Kjær artificial mastoid type 4930 using "acceleration"—calibration values from Flottorp (1972) (see also Dahm, 1973)

Diagnostically, the patients fell in two groups One group showed cohesive test re sults, i e normal otoscopy, positive Bing, normal middle ear pressure, and stapedial re flexes present In contrast, the second group was difficult to evaluate because of contradictory test results, i e normal otoscopy, normal middle ear pressure, negative Bing, and no stapedial reflexes In the latter group further audiological tests were done Fowler's ABLB, when possible, caloric testing, brief tone audiometry, X-ray tomography, exploration of the middle ear, and, finally, cochleography by means of an ear canal electrode (Salomon & Elberling, 1971)

During a five-year period 39 patients, 18 males and 21 females, were found according to the forementioned criteria The age ranged from 6 to 75 years average 42 6 years Twenty-five patients had binaural symmetrical hearing loss, 14 patients had monaural af fection A total of 64 ears were affected Besides the procedures used for selection and diagnosis, speech reception threshold (SRT) and discrimination loss (DL) with a monosyllable phonetically non-balanced word list were determined in all patients (Danielsen et al , 1971) In 58 ears Bing's test was done Impedance measurements were performed with a Madsen Electronic acoustic impedance bridge model ZO 70 The acoustic reflex thresholds were determined by contralateral stimulation in 61 ears In 3 patients it was pos

Table I *Results of the speech reception thresholds and discrimination loss for all ears examined*

	SRT	DL
Median	20	0
95% confidence	15–25	0–0
Range	0–45	0–25

sible to make Fowler's ABLB test and in 5 patients caloric testing and tone-decay test were performed Bilateral brief tone audiometry was performed in 4 patients and 8 patients were examined by X-ray tomography of the inner ear Three patients were subjected to cochleography and 4 patients had their middle ear explored, as the hearing loss was at first considered to be of a conductive type Three patients had tubes inserted because of chronic secretory otitis media

RESULTS

Anamnesis

Thirteen patients (33%) had a family history f hearing impairment, while 20 patients (51%) , ere without known predisposition The hearing losses had a duration of as little as 2 months to as long as 15 years (average about 12–14 years) Ten patients (25%) had noticed worsening of the hearing loss Seven patients (17%) complained of tinnitus, while no patients had vertigo

Objective findings

By otoscopy 3 ears (5%) were classified as simple glue ears After adequate treatment, the otoscopies were normal, but the audio grams continued to show hearing losses in the low frequency area The median air- and bone conduction thresholds are seen in Fig 1 showing an "air–bone gap' of the low frequency area Furthermore, the hearing is normal at 2 kHz and 4 kHz Table I shows the results of the SRT and DL values Table II shows the percentage of ears where the results of va audiological tests indicated conductive perceptive hearing loss In 52 ears (81%) middle ear pressure was within normal ra By contralateral stimulation the middle ear flex could be elicited in 44 ears (68%) ears at a threshold indicating recruitm Three ears showed recruitment with Fowl ABLB test which indicated cochlear dam In 2 siblings the X-ray tomography showed ner ear defects of the Mondini type the were normal The cochleography performe 3 patients revealed, in all 3, signs of hair degeneration but no signs of impairment in transmission system

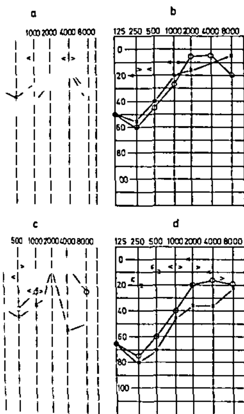
The patients who had their middle explored by operation due to an errone diagnosis will receive more detailed co ments

Patient 1

A girl, born 1957, first seen at the Otolo Department for tonsillectomy, 1969 At adm sion an audiogram was made which show bilateral air conduction thresholds at 40–50 HL in the low frequency area and normal bo conduction thresholds (Fig 2*a*) The pati underwent myringotomy, where middle ear fusion was found, removed and treated w tubes No hearing improvement occurred a the tubes were removed One year later peated audiological examination showed u changed hearing thresholds Bing's test w negative The middle ear pressure was norm A small stapedial reflex could be elicited contralateral stimulation with a Barany

Table II *Percentage of ears pointing towar conductive and perceptive hearing loss b otoscopy Bing's test, and acoustic stapedi reflex*

	Otoscopy	Bing's test	Acoustic reflex
Conductive	5%	25%	26%
Perceptive	95%	65%	68%



2 Audiograms of four patients (cases 1–4) with low ...ency sensorineural hearing loss who had their middle ...ored due to an erroneous preoperative diagnosis

The X ray tomography was normal ...as concluded that a probably slight ossi... ...ar fixation was present The right middle ...was explored and the stapes was palpated ...htly fixed, but no otosclerotic focus was The incus and malleus were mobile The ...ial footplate was mobilized, but no ... improvement occurred ...he patient was last seen in May 1975 The ... was normal The audiogram was ...letely unchanged Bing's test was still middle ear pressure −250/−200 mm ... pressure, small acoustic reflexes could be ...ited, and brief tone audiometry gave a conduction within the air conduction Cochleography performed in 1971 ...ed abnormality in the hair cells The pa... was successfully treated with hearing aids Conclusion The patient has a sensorineural hearing loss in the low frequency area

Patient 2

Girl, born 1955, first seen at this department in 1971 She complained of hearing loss lasting for 7–8 years Otoscopy was normal and the audiogram confirmed a bilateral hearing loss in the low frequency area (Fig 2*b*) Bing's test was negative, middle ear pressure normal, bilateral stapedial reflexes were present with no signs of recruitment and no intensity variation was found by Gelle's test It was concluded that the patient had a conductive hearing loss due to some loose connection in the ossicular chain and her right middle ear was explored The ossicles were found to be normal, but no definite hydrodynamic effect was seen in the round window upon movement of the stapes This finding was inexplicable The postoperative audiogram showed unchanged hearing The patient was last seen in 1972 and her hearing impairment was unchanged Treatment with hearing aids was unsuccessful Conclusion The patient has a sensorineural hearing loss in the low frequency area

Patient 3

Girl, born 1957 with hearing impairment of uncertain type from 10 years of age In 1971, when first seen at this department, the otological examination was normal and the audiogram was as shown in Fig 2*c* Bing s test was negative, middle ear pressure normal, no stapedial reflexes could be elicited, but intensity variation was seen by Gelle's test X ray tomography was normal It was concluded that the patient had malformation in the middle ear and this was explored The ossicles were intact, but by palpation the surgeon got an impression of a slight fixation of the stapedial footplate It was mobile, but gave no movement of the round window membrane, thus leading to stapedectomia *ad modum* Shea No hearing improvement occurred and when last seen in 1974, the audiogram showed ...olds unchange since the first

At

1971 The patient is successfully treated with hearing aids Conclusion The patient has a sensorineural hearing loss most pronounced in the low frequency area

Patient 4

A boy, born 1965, admitted to the department in 1972 because of otosalpingitis He had complained of hearing impairment for half a year The audiological examination revealed bilateral hearing loss for lower frequencies (Fig 2*d*) Middle ear pressure was normal and no stapedial reflexes could be elicited Bing's test was negative Otomicroscopy showed signs of fixed malleus syndrome His left middle ear was explored and some fixation of the ossicular chain was found The fixation was loosened, but only 10 dB HL improvement was obtained Due to the unsuccessful treatment, the patient was again admitted to the hospital for operation of the right ear On palpation the same impression of ossicular fixation in this ear was present The incus was removed and reinserted on the stapes with crus longum pointing downwards and crus breve under the malleus Thereafter, reflex was seen in the round window by movement of the stapes After the operation a hearing improvement of 10 dB HL was seen at all frequencies, but still a pronounced hearing loss was present in the lower frequency area When last seen in Oct 1976 the audiogram was unchanged The patient is successfully treated with hearing aids Conclusion The patient has a sensorineural hearing loss for lower frequencies combined with a slight ossicular fixation

DISCUSSION

The difficulties in distinguishing between true conductive hearing losses and low tone perceptive hearing losses can be ascribed to 1) both may have the same shape of the air conduction audiogram, and 2) misleading bone conduction measurements showing a false "air–bone gap", and 3) in the low frequency sensorineural hearing loss special pr arise from the contradictory informati tained by different audiological tests

As earlier mentioned, hearing loss fo frequencies is mostly seen when some fixation is present in the sound transı system showing a stiffness tilt But ported, the hearing loss may be due t kind of abnormality in the hair cells apical part of the cochlea (Schuknecht 1952, Vanderbilt University Hereditar ness Study Group, 1968) Some autho regard the patients as being completely the low frequency area (Gravendeel & 1960) They believe that the measured olds in this frequency area are answer harmonics, of which the higher freq consist, which may have sufficient inte be heard

As mentioned in the introduction, t conduction thresholds in some con hearing losses are measured too low s ing a perceptive hearing loss and in s neural hearing loss it is vice versa accuracy of bone conduction determina due to technical and biological phen In connection with perceptive hearing is relevant to mention just a few of the cal reasons for the invalidity of bone c tion The occlusion effect (Goldstein & 1965, Tonndorf et al , 1966), the jaw p (Schuchman & Burgi, 1971), and the window release phenomenon (Tonn Tabor, 1962) Furthermore, it has been that below 1000 Hz, harmonic disto tonal bone conduction stimulations distortion products only 20 dB bel levels of the fundamentals (Khanna 1976) Furthermore, an absolute sys error of bone conduction determinatio 15 dB may be present (Carhart & 1949) To avoid traditional bone con determinations, alternative methods used, such as the SAL-test or brief tone metry (Jerger & Tillman, 1960, Pede Salomon, 1977)

From a communicative point of vi

, will manage very well because the ability is preserved in the high fre area Due to this preservation, simple tests will not reveal this type of hear oss since whispered speech is heard than voiced speech (Ross & Matkin, When the patients for different reasons ' for examination, the contradic in information obtained by audiological is obvious from the fact that Bing's test the impedance measurements pointed ds conductive hearing loss in 25% of s The contradiction is also apparent the 4 referred case histories, where the ined otological and audiological findings surgical treatment on the basis of diag c errors In our opinion the diagnosis of orineural hearing loss in the low fre- cy area is often very difficult and must ly depend on the results of cochleo- hy (Elberling & Salomon, 1976)

the hearing loss is most pronounced in ow frequency area it may be diagnosed as iere s disease (Enander & Stahle, 1967) is also progressive and may occur at any (Sørensen, 1959, Harrison, 1962, Parving, None of our patients had vertigo, but %) had progression of hearing loss, only 2 of them complained of tinni Other authors have also found sensori hearing loss in the low frequency area different from the well known low fre- ncy hearing loss in Meniere's disease ima et al, 1967)

(1965) and Carhart (1962) be- 'the low frequency sensorineural hearing to be indicative of labyrinthine otosclero- The X ray tomography performed in our ients was normal except in 2 cases showing fondini defect, but in the absence of evidence it is not possible to exclude orphologic alteration of the organ of Corti possible cause of the hearing loss

material of low frequency hearing loss different etiological types One in ed type is well known as an autosomal ant inherited trait which means that the possession of a single abnormal gene is suffi cient for full manifestation of the trait (Nance & Sweeney, 1975, Nance et al, 1970, Nance, 1971) Another type due to cochlear malforma tion according to the Mondini type is probably also inherited, but our results permit no con clusion to be drawn on the mode of transmis sion A third group showed a mixture of audio logical findings, probably consisting of several different types as no certain entity could be outlined

When the diagnosis is fully established the patient should be treated with hearing aids, if the degree of hearing loss so indicates This treatment is often not successful, as the hear ing ability is normal in the high frequency area giving a good discrimination ability (Arians son, 1972) This good discrimination ability may be spoilt by distortion, acoustic feedback, and by other disadvantages of wearing a hear ing aid Moreover, most available hearing aids have a frequency characteristic and amplifica tion which is not suited to this type of hearing loss, although a few hearing aids with only low frequency amplification have been con structed As abnormal behaviour due to sec ondary psychological problems has been re ported in this type of hearing impairment, hearing aids should always be tried (Ross & Matkin 1967)

ZUSAMMENFASSUNG

Die otologischen und audiologischen Befunde bei 39 Patienten mit niederfrequent sensorischer Schwerhörig keit werden referiert Diese sensorische Schwerhörig keit ist schwierig zu diagnostizieren da die Audiogramm form ähnlich konduktiver Schwerhörigkeit ist und weil die Bestimmungen der Knochenleitung unsicher sind In niederfrequent sensorischer Schwerhörigkeit sieht man ein air–bone gap Dieses kann auf Grundlage eines fehlartigen Befunds zur Operation führen Die Schwer hörigkeit in Menierescher Krankheit ist ähnlich der niederfrequenten sensorischen Schwerhörigkeit sondern kein Schwindel ist hier anwesend Das Material zeigt daß verschiedene Typen von niederfrequent sensori scher Schwerhörigkeit unterschieden werden können 1) Der dominant erbliche Typ 2) ein Typ mit Malforma tion in dem inneren Ohr vielleicht auch erblich und 3) ein Typ mit verschiedenen und kontroversiellen audio-

logischen Funden Wenn die Befunde feststehen, sollen die Patienten mit spezial konstruierten Hörapparaten versuchsweise behandelt werden

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SOME EXPERIMENTS ON TEMPORARY THRESHOLD SHIFTS PRODUCED BY SHORT TONES

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tract TTSs produced by short (500–1 000 msec) pure uency (2 000 3 000 4 000 c p s) tones on normal ng subjects show under comparable conditions the ne essential characteristics as TTSs which follow ger stimulations In particular they show a tendency a displacement of their maximal values as soon as the mulat ng tone is presented at adequately high (100 dB L) intensity levels towards a frequency about a half tave higher than that of the stimulating tone In our ex riments however this tendency was more clearly and nstantly demonstrable following stimulations with 3 000 s tones S gnificant individual variations in quantita e aspects of the TTSs have been observed It is sug sted that the use of short tones may be easier and more *plicable* in practice in investigations concerning the er effects of acoustical stimulation and possibly also in ocedures aimed at obtaining a better understanding of e sign ficance of individual variations in the behaviour the receptor organ upon exposure to high intensity mulat ons

tudies concerning some after effects (tem orary threshold shift) which can still be de ionstrated immediately after the cessation of stimulation by *short* (500–1 000 msec) pure ones presented at increasing intensity levels ave been periodically carried out by many in estigators, as a way of obtaining information bout the functional behaviour of the receptor rgan

A survey of the most significant results by arly investigators, along with a personal con ribution was published in 1953 by O Bent- ʼen on the occasion of his doctoral disserta ion

The characteristic pattern shown by the threshold shift as a function of the intensity level of the stimulating tone and of the frequency of the test tone, is widely known In essence, at low and moderate intensity levels, the maximal threshold elevation occurs for a test tone of the same frequency as that of the stimulating tone Higher frequencies show a threshold shift gradually decreasing in magni tude as the frequency of the test tone increases, the extension of the affected area de pending on the intensity level of the stimulat ing tone Lower tones show practically no sig nificant threshold shift

It has been observed, however (among others by Zwislocki and by one of the present authors) that as soon as the intensity of the stimulating tone reaches high SP levels (90–100 dB) such a characteristic and monotonous pattern shows a remarkable tendency to significant changes

The maximal threshold shift is no longer clearly and typically confined to the frequency of the stimulating tone but shows a more or less pronounced tendency to reach a second maximum at a frequency about one half octave higher Fairly frequently the maximal threshold shift is clearly displaced to such a frequency

The behaviour of the threshold shift produced by low and moderate intensity tones seems to find a reasonable and easy interpretation as a state of residual excitation (residual

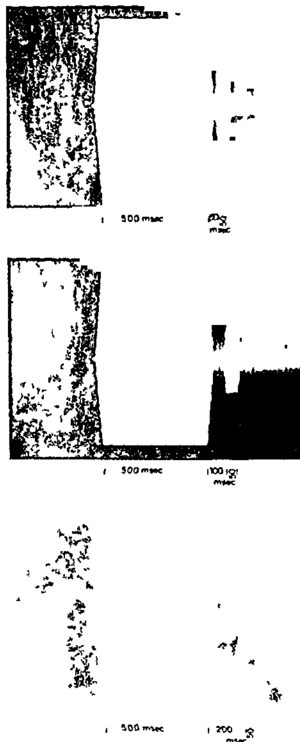


Fig 1 Sequences of tones as used in the different experiments

masking adaptation or related phenomena) It seems to represent a physiological process to reproduce in some way the pattern of the mechanical process of excitation and to be correlated with the arrangement of the ne- endings' distribution along the cochlear duct

The onset of a second maximum the displacement of the maximal threshold shift to a frequency one half octave or more above that of the stimulating tone can be explained on in terms of a new, different situation The obvious inference is that such a phenomenon may be linked to some sort of impairment of a certain number of functional units to be considered as a consequence although temporary, of the mechanical stress to which they have been submitted (Zwislocki & Pirodda 1952, Bentzen 1953 etc) The analogy with quite similar effects produced by longer stimulations obviously supports such an interpretation Zwislocki was able to give a mathematical analysis of the mechanical factors involved, and thus to find a theoretical explanation for this displacement of the maximum threshold shift, showing that there is a mechanical magnitude, in the process of excitation (the apparent acoustic power per unit length the cochlear duct) the local maximum of which is shifted, with respect to the place of maximum vibration, towards the base of the cochlea.

Even for short tones, furthermore the duration of the recovery time of threshold shift produced by high intensity levels is more than significantly longer than the few milliseconds needed for the restoration of the presumed fatory threshold when stimulations of low-to moderate intensity levels are involved

As a practical consequence the TTS produced by stimulations carried out at high intensity levels may still be clearly demonstrable when the pure residual excitation produced by low and moderate intensity levels has rapidly disappeared

Finally, even when short tones are used the magnitude and the duration of the TTS produced by high intensity stimulations show some relationship to the duration of the stimulus, which does not happen for a short duration after effects elicited by low and moderate intensity levels

In short, the qualitative similarity between

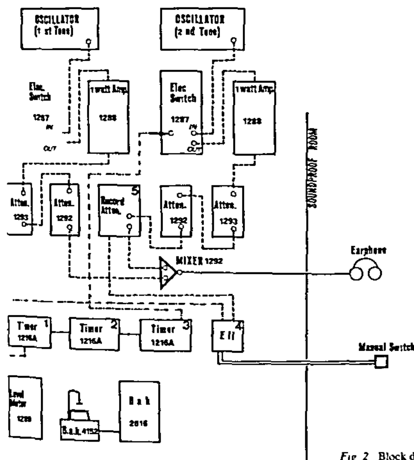


Fig 2 Block diagram of the equipment

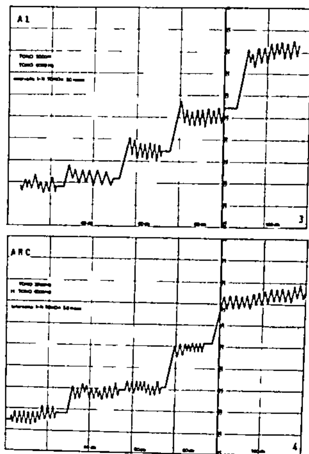
-r-effects produced by short stimulations l effects produced by long stimulations is nplete

It is well known that much work has been ne on the generally accepted assumption at some kind of correlation exists between TS and PTS produced by exposure to noise Practical application of the general principle hich can hardly be overlooked) to the in vidual situation and to the prediction of in- vidual reaction to exposure to noise is, how er, highly controversial (Ward et al , 1959, .rns & Robinson, 1970, among many others) d subject to many uncertainty factors

Consequently, we felt that further experi- -nts on the behaviour of after-effects follow- g stimulations by short tones would be justi -d, with the aim of collecting additional data and in the hope that some day, in some way, they may be useful in working out a reliable clinical application of such procedures for the evaluation of individual susceptibility to acoustic overloading The use of short tones should provide some advantages a shortening of the test time, and perhaps a safer and more easily accepted experimental condition than those corresponding to procedures involving longer stimulations

SUBJECTS EQUIPMENT, EXPERIMENTAL PROCEDURES

Since one of the primary purposes of the present investigation was to collect highly reliable data (many discrepancies are to be found in



Figs 3–4 Some samples of threshold tracings obtained from tested subjects Note the tendency to a progressive ıncrement of the TS at the 100 dB ıntensıty level

the findıngs of prevıous and especıally early ınvestıgators, whıch seem ın most cases to be attrıbutable to technıcal factors) experıments were carrıed out on a small group of 20 selected, young (under 30 years) healthy, normally hearıng and well traıned subjects

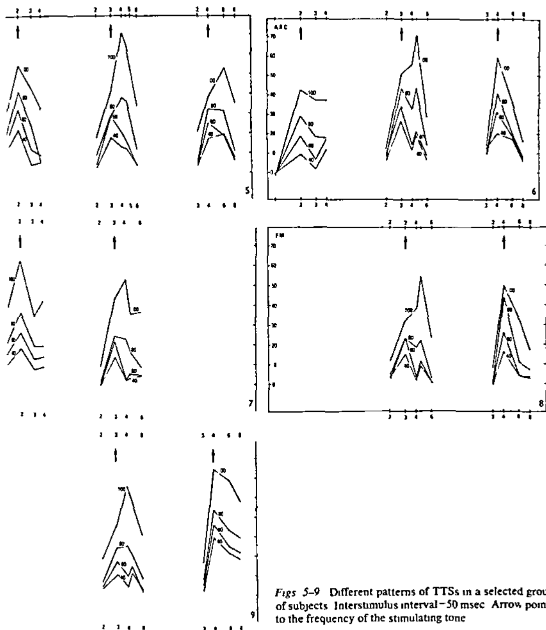
Only pure frequency tones of 2000 3000 and 4000 c p s were used as stımulatıng tones Theır duratıon was eıther of 500 or of 1000 msec durıng dıfferent experımental sessıons The rıse–decay tıme was kept wıthın values (10–25 msec) from whıch no sıgnıficant ınterference wıth experımental results was to be expected The ıntensıty levels of the stımulatıng tone used ın the present experıments were 40, 60, 80 and 100 dB (SPL)

The threshold shıft was determıned by a test tone of 50 msec duratıon (rıse–decay t msec) (Fıg 1) In order to achıeve a p nary orıentatıon, and to try to reduce a as possıble the duratıon of the test proced a lımıted number of frequencıes was te the attentıon beıng focused on the frequ one half octave hıgher than that of the st latıng tone On the basıs of prevıously tablıshed facts, the ınterval between the s ulatıng and the test tone was kept constan msec peak-to peak) ın the majorıty of the perıments on some subjects however ı vals of 100 and 200 msec were used on ı sumptıon, as mentıoned before that o loadıng phenomena should have show longer persıstence, thus allowıng a better easıer demonstratıon of theır presence aga a more rapıdly dısappearıng short dura threshold shıft due to resıdual adaptatıon addıtıon, a longer ınterval between the tones should make easıer the determınatıo the threshold shıft, partıcularly for untraı subjects

The block dıagram (Fıg 2) shows the sentıal characterıstıcs of the equıpment w has been used Checkıng of functıons of ferent unıts and frequency and amplıtude c bratıons were repeated accurately before after every experıment sessıon

In order to achıeve the best accuracy ın measurement of the threshold shıft the th hold trackıng procedure has been used the mean value calculated for each ınten level taken as the final value A Grason St ler recordıng attenuator model E3262 had be modıfied for our experımental requıreme ın order to elımınate the on and off-effe generated by the relays whıch produce dıstortıon ın the stımulatıng tone

Such a procedure ımplıed of course repeated presentatıon of the sequence of two sıgnals at any ıntensıty level of the st ulatıng tone Each sıngle traın was trıgger by the tested subject hımself by operatıng key, at any desıred moment when he felt p pared to concentrate on the task of makıng very careful and attentıve judgement The



Figs 5–9 Different patterns of TTSs in a selected group of subjects Interstimulus interval – 50 msec Arrow points to the frequency of the stimulating tone

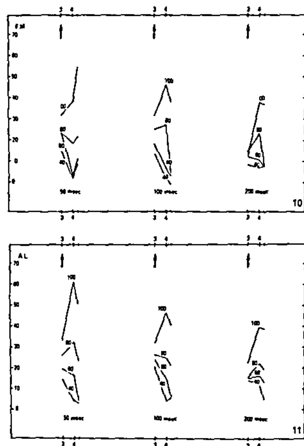
ᵈ splacement of the key elicited an incre-
ent the downward displacement a decre
ent by 1 dB of the intensity of the test tone in
corresponding sequence of stimuli

By this procedure, the tested subject was
..d to make a decision every time he trig-
ᵗ train of signals, whether he heard the
tone or not, and decide, consequently
hether to increase or decrease its intensity in
successive sequence

Some examples of traces obtained by this procedure from different subjects are given in Figs 3 and 4

RESULTS

Experiments, even on young trained subjects, proved to be more time-consuming and their results somehow less predictable than ex-



Figs 10–15 Comparison among TSs as measured at 50 100 200 msec after the cessation of the stimulating tone The displacement of the maximum threshold elevation to a frequency a half octave higher is always demonstrable

ted on the basis of previous research herefore, only preliminary results can be given at the present

Some essential findings seem to emerge with unquestionable evidence from the experiments which have been performed up to the present time, namely

(1) In contrast to some previously accepted views, in agreement with others, the shift of the maximum threshold elevation to higher frequencies, and especially to a frequency about one half octave higher than that of the stimulating tone, does not occur as a constant phenomenon and in any case does not show the same pattern for any frequency of the stimulating tone, as shown in the graphs which illustrate individual findings from some selected subjects (Figs 5–9)

Even in our experiments the displacement of the maximal threshold shift could be observed almost constantly, and sometimes with impressive quantitative characteristics when the frequency of the stimulation tone was 3000 c p s (Figs 5–9) which is in agreement with the results of experiments carried out by employing longer stimulations Once again, even when after effects following short tones are concerned, the area of the cochlear receptor corresponding to the frequencies of 4000–4500 c p s shows a critical sensitivity to mechanical damage

Such a behaviour must be submitted to further investigation especially, in our opinion, as regards its variability in one and the same individual It should represent an useful indication in view of a possible application of these procedures to clinical purposes

(2) The magnitude of the observed threshold shifts for frequencies higher than that of the stimulating tone has shown even in our experiments, a very *wide individual variability* (Figs 5–9)

(3) The repetition of the stimulation even at relatively long time intervals when the stimulating tone was presented at the highest intensity level (100 dB), occasionally produced some kind of cumulative effect a tendency to a progressive increment of the threshold shift as demonstrated by the ascending pattern of the corresponding trace (Figs 3–4) from individual cases

After effects following stimulations by short tones in other words, behave, from a qualitative point of view, in exactly the same way as temporary threshold shifts produced by longer stimulations

In the present situation therefore they should receive greater consideration when planning further research on the intriguing and complex problem of the TTS/PTS relationship The study of their behaviour could provide a means of obtaining highly reliable information by a relatively simple and time sparing procedure, easy to perform under routine clinical conditions

ZUSAMMENFASSUNG

porare durch kurze (500–1000 msec) Reiztone rei Frequenz (2000–3000–4000 Hz) verursachte Schwel en weisen bei vergleichbaren experimentel Bedingungen ein vollkommen analoges Verhalten diejenigen nach langeren Stimulationen erhaltenen Die maximalen Werte solcher Schwellenerhohungen anderem zeigen sobald der Reizton ein genugend (100 dB SPL) Intensitatsniveau erreicht hat, eine liche Tendenz zur Verschiebung nach einer Fre die etwa eine halbe Oktave hoher als die Frequenz Reiztones liegt Auch bei unseren Befunden konnte diese Tendenz besonders klar und konstant nach esen werden nur wenn die Schwellenerhohung inen Reizton von 3000 Hz verursacht worden war llenveränderungen zeigten bei den verschiedenen bedeutsame quantitative individuelle Varia Kurze Tonreize können mit einigen Vorteilen gewinn Genauigkeit der Messungen) der weiteren ichungen uber Nacheffekte starker akustischen ze angewandt werden Eine Erklärung uber die Be tung individueller Variationen konnte klinische An ingen mit sich bringen

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VASCULAR HISTOLOGY OF THE GUINEA PIG COCHLEA

A Axelsson and D Vertes

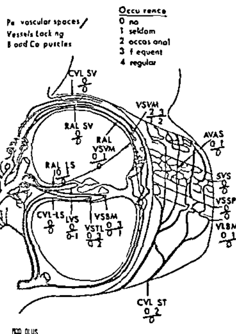
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Abstract Detailed histological findings on all the regularly occurring cochlear vessels in the normal guinea pig are presented Vessels were examined using a surface preparation technique and phase contrast microscopy Particular attention was paid to the density of red blood corpuscles the thickness of the vascular wall the occurrence of endothelial cells and pericytes together with their possible influence on the vessel lumen and the existence of perivascular spaces and vessels lacking blood corpuscles The data are compared with some previous findings of vascular pathology ' following noise stimulation The possible functional significance of some of the morphological findings is also discussed

The study of the cochlear blood supply is sometimes limited by its complexity and inaccessibility The location of vascular pathology is for example, subject to change, depending on individual susceptibility differences, variability between different vessel systems, as well as differences in the vasculature between the cochlear turns Although this variability seems to suggest a need for systematic investigations in which all cochlear vessels in control animals as well as in experimental animals are examined, a review of the literature reveals a lack of such studies It is not surprising, then, that the role of the blood supply in noise induced hearing loss is not clearly understood Some authors reportedly found a decrease in red blood cells in the vessels below the basilar membrane (Lawrence et al , 1967, Lipscomb & Roettger 1973) and in the stria vascularis (Lawrence, 1972) following noise exposure Presumably related to this was the presence of swollen endothel al ce partially occluding the vessel lumen Wh both endothelial cells and pericytes a thought to be involved in the regulation blood flow (Rhodin, 1968, Kellerhals 197 Kimura & Ota, 1974), the mechanism of s regulation is still disputed Kellerhals (19 b) while noting no changes in the spiral lam capillary networks, found some strial ves packed with red blood cells following posure to impulse noise Duvall et al (1 sacrificed animals at various intervals foll ing noise exposure, they reported change the stria vascularis ranging from constri and collapsed vessels containing few red bl corpuscles, to dilated vessels filled with d torted red blood corpuscles The density red blood cells, whether packed or wide spaced, could also be a result of the prepa tion technique (Lawrence, 1972, Axelss 1976) Fried et al (1976) exposed chinch to intense low frequency octave band no In most of their animals they found one two areas of complete degeneration of the s vascularis They found inconsistencies in location and extent of such damage Usi similar low frequency octave band noise a high frequency octave band noise as w Bohne (1976) reported minimal damage to blood vessels beneath the maximally dama

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FOD PLUS

ENDOLYMPH AND ASSOCIATED VESSELS

PERILYMPH AND ASSOCIATED VESSELS

1 One cochlear turn apico-basal section showing larly occurring vessels their relationship to cochlear s and the existence of perivascular spaces (*PVS*)

SVM radiating arterioles – scala vestibuli basally -SV collecting venules – scala vestibuli VSVM the el at the vestibular membrane AVAS arteriovenous tomoses *SVS* stria vascularis *VSSP* the vessel of prominence *VLBM* the venules at the basilar ane *CVL–ST* collecting venules – scala tympani -*LS* radiating arterioles – spiral lamina *CVL–LS* ing venules – spiral lamina *LVS* limbus vessels L the vessel of the tympanic lip *VSBM* the vessel lar membrane

ions of the organ of Corti but the condi of other vessels throughout the cochlea not described Thus, although the blood ly is increasingly thought to have an in- e in various types of sensorineural hear- losses, its influence in noise trauma is ...stal Too often results have been in d as representing pathology—without ear basis of normative data with which e results could be compared

It is the purpose of this study to begin to supply some of that normative data by which vascular pathology, regardless of cause, might be assessed

MATERIAL AND METHODS

Seven normal young healthy guinea pigs were used for the experiments The animals were sacrificed without anesthesia by decapitation with sharp scissors The temporal bones were immediately removed, the bullas opened and the cochlea assessed according to a previously described method (Axelsson et al, 1974 1975) Briefly, the cochlea was fixed by slowly injecting 5% glutaraldehyde through an apical opening and through the oval window from which the stapes had been removed After 24 hours in the fixative the cochlea was decalcified in 5% EDTA which was changed daily until the decalcification process was complete (about 7 days) An apico-basal section of the cochlea was made and all structures were examined for gross changes The cochlea was then counterstained in 0 05% osmic acid for 10 minutes, dehydrated in an alcohol series of increasing concentration and stored in glycerin In one animal the osmic acid counterstaining of both ears was omitted in order to evaluate the influence of this stain on cochlear vessels After further dissection cochlear specimens were examined by phase contrast microscopy usually under 250–1 200× magnification The following regularly occurring cochlear vessels were examined in all half turns of each cochlea of each animal

External wall

Scala vestibuli
radiating arterioles
collecting venules
the vessel at the vestibular membrane
the vessel of the scala vestibuli

Scala media
radiating arterioles
arteriovenous anastomoses
stria vascularis
the vessel of the spiral prominence

Scala tympani
the venules at the basilar membrane
collecting venules

Spiral lamina
radiating arterioles
collecting venules
the limbus vessels
the vessel of the basilar membrane
the vessel of the tympanic lip

A schematic diagram of these vessels including their relation to the cochlear lymphs is shown in Fig 1 In all these vessels particular attention was paid to the following (1) the distance between red blood corpuscles (RBC), (2) the thickness of the vascular wall, (3) the occurrence and size of pericytes (PC) and endothelial cell nuclei, hereafter referred to as endothelial cells (EC), and their possible influence on the vessel lumen, (4) the existence of so called perivascular spaces (PVS) and vessels lacking blood corpuscles (VSLBC) the formerly called avascular channels, and (5) the occurrence of pigment granules, melanocytes or any other regularly present extracellular substance in the stria bed

RESULTS

Radiating arterioles of the Scala vestibuli (RAL–SV)

The radiating arterioles of the external wall were studied apically where the scala vestibuli merges with the scala tympani of the next turn This is the region immediately peripheral to the so called glomeruli , the winding parts of the RAL with a spring coil appearance The number of RAL in this region is high, particularly in the basal turn The RAL run radially over the scala vestibuli to supply all capillary areas in the external wall Red blood corpuscles (RBC) appeared in the lumen in several rows and were situated close to each other The vessel wall was always thick Due to this thickness, endothelial cells (ECs) and pericytes (PCs) were difficult to observ casional PCs were observed ho ticularly in the second and fourth t appeared to be of normal size ar no influence on the vascular lumen casional perivascular space or vessel blood corpuscles could be obser fourth turn of the cochlea, but this unusual finding

Radiating arterioles of the Scala basally (RAL–VSVM)

The radiating arterioles of the scala were also examined in the basal part external wall at the level of the vessel vestibular membrane (VSVM) The lumen in all turns was filled by RBCs lay close to each other and were oriente different planes Though still of conside size, the vascular wall was thinner than apically in the scala vestibuli especially a apical turns A slim layer of substance often seen on the outside of the vessel In many preparations ECs could often be served in the RAL at this level as a re finding Occasional PCs most commonly at the ramification of the vessel were served with a somewhat increasing frequ apically in the cochlea Perivascular sp were demonstrated in the three basal with a fairly low frequency There seen be a gradual transition from thick musc walled vessels to those with perivas spaces and thinner walls containing fewe elements The occasionally seen vessel ing blood corpuscles was most comm branches connecting VSVM In ge neither ECs or PCs appeared to influenc vessel lumen

Collecting venules of the Scala vestibuli (CVL–SV)

Occasional CVL run spirally in the most a parts of the scala vestibuli at the juncti two cochlear turns More peripherally scala vestibuli CVL often turn off at right angles and run radially and paral

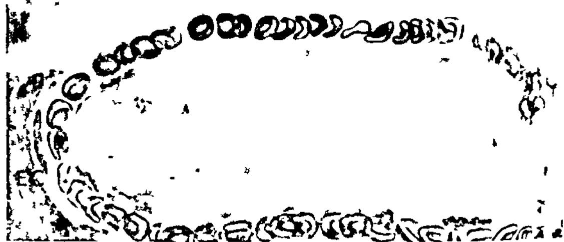


Fig 2 Guinea pig 4th turn external wall The capillaries of the stria vascularis (*SVS*) the vessel at the vestibular membrane (*VSVM*) and the vessel of the spiral prominence (*VSSP*) and a vessel lacking blood corpuscles (*VSLBC*) are seen The enclosed portion of VSVM is seen at a higher magnification in Fig 3 Arrows = remnants of dark material within VSLBC and the so-called blebs in SVS

L In general, RBCs were situated close each other and seemed to course easily y through the lumen Occasionally, lumen was narrower and blood cells were obliquely or longitudinally CVL had onsiderably thinner vascular wall than L Slim to normal sized ECs and normal- 1 PCs without influence on the vascular were a fairly frequent finding in all hlear turns In general no perivascular ces or vessels lacking blood corpuscles e demonstrated

vessel at the vestibular membrane VM)

VM shown in Figs 2–4, is a capillary vesl belonging to the capillary area apical to the attachment of the vestibular membrane in the external wall This capillary area is bordered by the vessel of the scala vestibuli (VSSV) apically and by the vessel at the vestibular membrane (VSVM) basally Usually RBCs of VSVM were situated close to each other and were situated longitudinally in the lumen Though less frequent, obliquely or trans versely oriented RBCs were also seen as shown in Figs 2 and 3 In general, the lumen appeared light, though a darkened lumen was occasionally seen It was not possible to determine if this was due to ECs or to other changes in the vessel wall or within the lumen The vessel wall appeared thin ECs were common and of normal shape Sometimes, as in Figs 3 and 4 they appeared to influence



Fg 3 Gu nea p g 4th turn vessel at the vestibular mem brane (*VSVM*) Well-def ned perivascular spaces (*PVS*→) endothelial cells (*ECs*) and pericytes (*PCs*) are observed Note the change n orientat on of red (→) at the s te of an EC juxtaposed w th a PC

the vascular lumen Normal sized PCs ap peared occasionally in the basal turn and at times in the apical turn Regardless of size PCs by themselves tended to have no dimin ishing effect on the vessel lumen Perivascular spaces were a regular finding but were som times absent or minimal towards the cochl base In no vessel other than VSVM and t vessel of the basilar membrane were the pe vascular spaces so easily observed Mostly



Fg 4 Gu nea p g 3 d turn ves el at vest bular membrane (*VSVM*) Uppe sel sect on shows that blood corpusc e be s tuated some d stance apart a fi eas ly m staken for a vessel lack ng bl corpuscles Arrow EC and adjacen appear to influence the vessel lumen

ared as in Fig 3, as a smooth, double our without any pursings Vessels lacking d corpuscles were also common in all s and appeared to be in direct commu tion with the perivascular spaces Most of vessels lacking blood corpuscles had a hazy grey and empty appearance (see 2) Sometimes, however, as is also shown ig 2, there seemed to be dark material ig what had previously been the lumen isitions from vessels lacking blood cor- les to fibrous strands were uncommon iously the lack of clear contrast from the orting tissues made vessels lacking blood iuscles with such a fibrous organization cult to discern

vessel of the scala vestibuli (VSSV)

vessel of the scala vestibuli, VSSV, which stitutes the apical margin of the capillary of the scala vestibuli, appeared similar to VM RBCs, however, were at times widely ed lying more than 5 μm from each other vascular spaces and slim PCs were some s present

a vascularis (SVS)

capillaries of the SVS (see Fig 2) showed t variation of all cochlear vessels between is in the same animal and between animals capillaries were always packed with -s The vascular wall appeared very thin no perivascular spaces were found The meter of the capillaries varied greatly and etimes segments of the capillaries would seen narrowing down and being trans ned into a vessel lacking blood corpuscles general neither PCs nor ECs could be ob ved though on rare occasions a long, thin tched EC or some kind of deposit was n in the walls of the capillaries Blebs or small circular empty spaces 5–6 μm in meter seen in Fig 2, were sometimes seen the vessel lumen in the second to fourth is Minute granula were often oriented ng vessels in all turns Larger clumps of ment were also a common finding through- out the cochlea Occasionally, melanocytes and pigment clusters, transitional forms of melanocytes, were seen Granula, pigment clumps and melanocytes all appeared to be more frequent apically As seen in cross sec- tion, it appeared that the pigment clumps were most deeply situated in the stria vascularis, with a layer of fine granula more superficially and a mixture of pigment clumps, fine granula and melanocytes closest to the SVS surface

The vessel of the spiral prominence (VSSP)

VSSP (see Fig 2) lies deep in the spiral prominence and consequently was less easily observed than many other vessels RBCs were situated close to each other, passing the lumen transversely and occasionally obliquely or longitudinally The vessel wall appeared thin and sometimes the vessel seemed to be sur- rounded by a thick tissue strut or collar Peri vascular spaces were not regularly found but what appeared to be a narrow perivascular space could at times be demonstrated in the basal turn Vessels lacking blood corpuscles were not demonstrated Though improving somewhat apically, ECs and PCs were typi cally difficult to observe When they were seen, they appeared to be small and slim PC influence on the vessel lumen could not be determined It appeared, however, that ECs often influenced the vascular lumen in that the blood corpuscles at these sites took a longi tudinal instead of a transverse course Melano- cytes without any direct relation to VSSP appeared in all turns but clearly less often in the basal turn than in the others

Radiating arterioles of the scala media (RAL–SM) and arteriovenous anastomoses (AVAS)

Branches of RAL–SV supply all kinds of capil lary areas in the external wall of the cochlea and lie external to the capillary vessels of the scala vestibuli and scala media Many branches run directly over to the collecting venules of the scala tympani without supplying any of the capillary areas These vessels ap-



Fig 5 Gu nea p g 1st turn o venule scala tympani Pocke l ke lar perivascular spaces (⬅) and tr pericytes (*PCs*) are seen Blood cell t on w th n lumen seem ngly alt endothel al cells (→)

pear to constitute some kind of by pass mechanism and are here termed AVAS This term while signifying that these vessels connect arterioles with venules may be incorrect from a histological point of view In view of their proximity to the SVS Fig 1 depicts the AVAS as endolymph facing vessels However based on evidence that the basal cells of the stria probably provide a tight seal between strial and spiral ligament vessels (Kimura & Schuknecht 1970 Smith 1973 Hawkins 1973) it is much more likely that the AVAS are associated with perilymph Blood corpuscles

within the lumen could be situated cl each other or some microns apart B RBCs were most often oriented transv within the lumen and apically most ofter tudinally Due to their location deep spiral ligament the existence of periva spaces PCs and ECs was difficult to ob Perivascular spaces were an unusual f sometimes it appeared that they were l in large calibre AVAS while present in of more delicate size Vessels lacking corpuscles were never seen in the A When observable ECs appeared fair



Guinea pig 1st turn the vessel of the tympanic STL) and the vessel of the basilar membrane M) The density of red blood corpuscles in VSBM n to vary from close to spaced Perivascular spaces (⇨) and vessels lacking blood corpuscles (➡) are seen in both vessels Channel like system of bridges between perivascular spaces and vessel lacking blood corpuscles in VSBM (⤻) are seen

it PCs infrequent, both of normal size and out any influence on the vascular lumen

ecting venules of the scala tympani L-ST) and venules at the basilar ibrane (VLBM)

((see Fig 5) drain all capillary areas in external wall They also connect directly the AVAS, particularly in the basal turn L constitute the only vessels in the scala pani of the external wall At the attach it of the basilar membrane, CVL make an ga formed loop and then, more basally, n run spirally for a short distance These tions, when viewed perpendicularly, seem constitute spirally running vessels, the ules of the basilar membrane (VLBM) histology of VLBM is very difficult to luate because of their location deep in the ral ligament The observations were similar those of the CVL-ST The RBCs in the nen of the CVL were situated close to each er and were oriented transversely In larger L there was more than one column of Cs often with obliquely or longitudinally ented RBCs It was very difficult to deter ne if there were perivascular spaces in these ssels and there was a great deal of individ ual variation As seen in Fig 5, straight peri vascular spaces similar to those of the vessel at the vestibular membrane and the vessel of the basilar membrane were not seen In the two basal turns there appeared to be some kind of pocket like irregular spaces surround ing CVL, particularly at the basal part of each scala tympani Apically, it was more common to find CVL without any perivascular like structures Vessels lacking blood corpuscles were never observed Normal sized PCs with out any influence on the vascular lumen were a common finding As opposed to other re gions of the cochlea PCs often appeared to have a triangular shape (see Fig 5) The fre quency of PCs seemed highest in the middle region of the cochlea and less frequent to-wards the base and apex ECs of normal ap-pearance and without influence on the vessel lumen were also a regular finding As shown in Fig 5 occasionally an EC juxtaposed with a PC was seen to influence the vessel lumen

Radiating arterioles and collecting venules of the spiral lamina (RAL, CVL-LS)

These vessels originate from the same central parts of each cochlear turn and radiate over the spiral lamina They are fairly easy to dif



Fig 7 Guinea pig 2nd turn the vessel of the tympanic lip (VSTL) and the vessel of the basilar membrane (VSBM) Granula surround VSBM Perivascular spaces (➡) are present in VSTL but are lacking in VSBM ... dothelial cell appears to diminish vessel lumen (→) ...

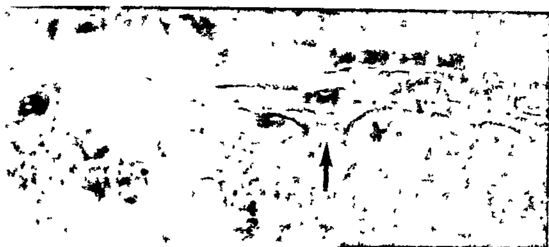
ferentiate when contrast injected but impossible to separate with the present technique In addition these vessels are very hard to discern due to the osmic acid stained nerve fibres which obscure the view In one animal in which the cochlea was unstained these vessels were easier to observe RBCs were transversely situated in the lumen There appeared to be large perivascular spaces in many areas no vessels lacking blood corpuscles and frequent PCs and ECs of normal appearance Although EC influence on the vessel lumen could not be determined sometimes a large PC appeared to reduce the lumen at the level of attachment to the vestibular membrane

The limbus vessels (LVS)

LVS are made up of capillary arcades in the spiral limbus and are the only vessels of the spiral lamina which face endolymph (see Fig 1) They are situated fairly deeply in the tissue and consequently it is difficult to get a close view of them Most commonly RBCs in the lumen lay close together but more widely spaced RBCs could also be seen ... blood cells were transversely oriented in ... lumen or, less commonly obliquely In ... eral perivascular spaces and vessels ... blood corpuscles were not demonstra... When found vessels lacking blood corpus... tended to be in the basal parts of the co... lea PCs were an infrequent finding their ... was generally normal but in some coch... they were slim They did not influence ... vessel lumen ECs of normal appearance ... with no diminishing effect on the lumen w... frequently seen

The vessels of the basilar membrane (VSBM)

VSBM also called vas spirale or the ... spiral vessel is shown in Figs 6–8 This ... interesting vessel since it is most cl... situated to the organ of Corti (OOC) an... regularly found in man as well as in gu... pig In other mammals it appears to be r... mentary and is only occasionally dem... strated (rhesus monkey chinchilla) or is



* Guinea pig 2nd turn the vessel of the basilar (*VSBM*) The density and orientation of red blood corpuscles are seen Arrows—tube like openings from perivascular spaces (*PVS*)

all (rabbit) In the guinea pig, VSBM most easily observed in the second half first turn The diameter of VSBM appeared to vary appreciably and consequently density of the RBCs also varied In general, RBCs were situated close to or within microns of each other Occasionally, there would be larger segments of the vessel with much greater separation between RBCs The RBCs were almost invariably situated nally in the lumen and very seldom transversely or obliquely Perivascular spaces were regularly demonstrated in the basal, seldom in the second and only occasionally in third and fourth cochlear turns Compare, for example, Figs 6 and 7 showing turns I 0 and I 5, respectively Vessels lacking blood cles were not a frequent finding in any but were most often demonstrated in the and least often in the third turn PCs more or less regularly found and were arly more common in the apical two turns the cochlea PCs of larger than normal size were observed in all except the basal turn with fairly equal distribution PCs did not appear influence the vessel lumen diameter ECs a frequent finding in all cochlear turns ugh not usual, ECs in the two basal turns appear larger than normal (as in Fig 7) and cause a slight depression in the vascular wall Granulated material, also seen in Fig 7, regularly surrounded VSBM particularly in the two basal turns The origin or character of these granula is unknown, however they were not as easily observed in the unstained animal thus indicating that they may be osmiophilic in character As is seen most clearly in Fig 6, perivascular spaces often formed a separate, almost channel like system of bridges and open spaces together with the vessels lacking blood corpuscles A finding most common in the first turn which has not previously been demonstrated was what appeared to be openings like small tubes from these perivascular spaces into the tissues (see Fig 8) These appeared both on the peripheral and on the central side of VSBM but were more common on the former ECs on the vessel wall opposite to the perivascular tube openings were common

The vessel of the tympanic lip (VSTL)

As shown in Figs 6 and 7, osmic acid darkens the myelinated nerve endings, thus making VSTL or the inner spiral vessel, difficult to observe in preparations treated with the present technique Though RBCs were most commonly situated close to each other, there

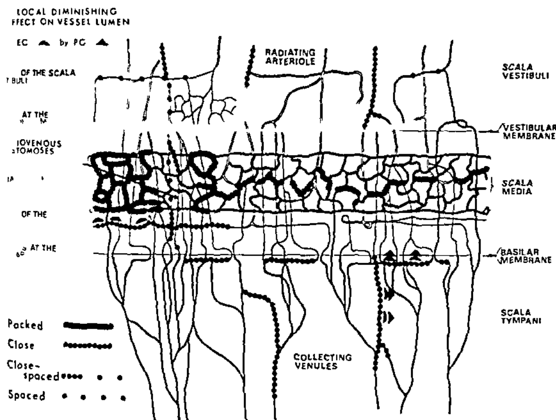
large variations and often wider-spaced RBCs were seen In general, RBCs were oriented longitudinally in the lumen, occasionally obliquely or transversely. Perivascular spaces were always found in the basal turn and decreased apically The perivascular spaces often appeared to be larger than those surrounding VSBM Short segments of vessels lacking blood corpuscles were occasionally demonstrated PCs, though not abundant, were demonstrated with increasing frequency in the apical turns ECs which were less frequently seen on this vessel than on VSBM, sometimes appeared to influence the vascular lumen The granulated material surrounding VSBM was in general not demonstrated around VSTL but sometimes followed an interconnection between the two vessels and continued for a short distance around the VSTL

DISCUSSION

The advantages and disadvantages of our modified method of viewing the vasculature have been described previously (Axelsson et al, 1974, 1975) We are aware that the present technique may influence the cochlear vessels in that circulation and vessel function does not stop instantaneously with the death of an animal We are equally aware that this technique results in a static view of a normally dynamic process Such limitations, however, are inherent in all presently available techniques

Some cochlear vessels are highly variable between animals, while others, notably the capillaries of the stria vascularis, appear similar in different individuals Vessels and related vascular phenomena can also vary between ears in the same animal, between turns of a single cochlea, and between sections of the same turn The descriptions which follow, therefore, represent the generalized findings of a large number of observations Exceptions and variations, however, may and do occur—thus the data presented should not be thought of as definitive In addition most of the observations described here are "visual impr- sions", and, as such, cannot conclusi- determine the existence of, for example pe- vascular spaces and/or vessels lacking bl- corpuscles Obviously, other histological tec- niques employing cross-sectional views of v- sels are necessary for these types of det- minations

A summary of cochlear vessels facing pe- lymph and those facing endolymph is sho- in Fig 1 This figure also shows interestin- that perivascular spaces are most commo- found and most regularly occur in vessels f- ing perilymph These vessels include the f- lowing Radiating arterioles of the scala ve- buli basally, collecting venules of the sc- vestibuli, the vessel at the vestibular m- brane, the vessel of the scala vestibuli rad- ing arterioles and collecting venules of spiral lamina, the vessel of the basilar m- brane and the vessel of the tympanic Those vessels facing perilymph which do have regular or frequent perivascular spa- are radiating arterioles of the scala vesti- apically, with their thick vascular walls the sparsely occurring collecting venule- the scala vestibuli Perivascular spaces also irregular and/or infrequent in the vascularis, the vessel of the spiral promine- arteriovenous anastomoses and the lim- vessels We feel that the occurrence of p- vascular spaces in vessels facing perily- and not in those facing endolymph must b- functional significance The most prob- explanation would be that these spaces tain some kind of intermediate fluid betw- blood and perilymph It has been specul- (Maggio, 1966, Schindler & Schneider that the radiating arterioles of the scala v- buli are one possible source of perilymph duction The fact that the radiating arter- of the scala vestibuli apically have few gular perivascular spaces does not, of cou- refute this It does suggest, however, perilymph transfer and absorption is r- likely to occur in the radiating arterioles ther peripherally In general, no opening c-



9 One cochlear turn external wall, showing the sity of red blood corpuscles and the influence of endolial cells (*EC*) and pericytes (*PC*) on the vessel lumen

found on the perivascular spaces at the lopted magnifications Only in the vessel of e basilar membrane were there sometimes l tube like openings (see Fig 8) on both e central and more frequently the peripheral These give the visual impression of an hose pouring perivascular fluid out in the sues and fluids nearby, i e myelinated rve endings, the organ of Corti and perimph Alternatively, these hose-like strucures could be fibrous strands of vessel walls rtainly, it is beyond the scope of this study determine the exact nature of these pericular elements Conclusive evidence must wait electron microscopic investigations

With the exception of an occasional occurrence in the limbus vessels, vessels lacking lood corpuscles were also only found in the owing vessels which face perilymph the vessel at the vestibular membrane, the vessel of the basilar membrane and the vessel of the tympanic lip (see Fig 1) Particularly in the spiral lamina, there is a clear communication between the perivascular spaces and the vessels lacking blood corpuscles As is shown in Fig 6, it appears that the vessels lacking blood corpuscles, in some cases, are part of an extraluminal vascular fluid system Contrarily, the hazy grey vessels lacking blood corpuscles in the scala vestibuli of the external wall (Figs 2 and 4) appear more as possibly degenerated vessels The ultimate question whether vessels lacking blood corpuscles function in the circulation of a non-corpuscular fluid such as plasma or are closed-down vessels cannot be solved by the present technique

As stated in the introduction of this paper, the previously termed 'avascular channels'

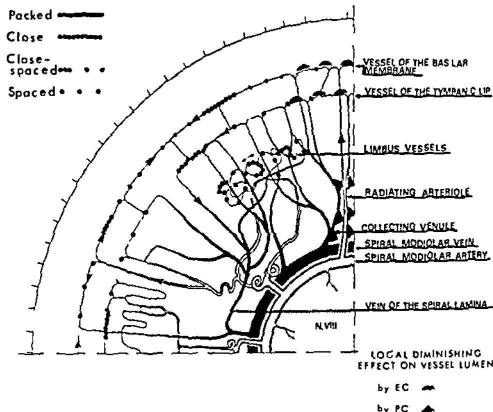


Fig 10 One cochlear turn spiral lamina showing the density of red blood corpuscles and the influence of endothelial cells (*EC*) and pericytes (*PC*) on the ve lumen

have here been called 'vessels lacking blood corpuscles' The term avascular indicates that is no circulation of blood elements The of a vessel without RBCs does not necessarily indicate such a state, since this vessel or vessel section may be circulating either plasma (plasma skimming) or serum Another possibility is that there is a momentary or somewhat longer interference with this vessel or vessel section because of a transient blockage by a red or white blood corpuscle These channels are certainly vascular, though with the present techniques it cannot be stated whether there is circulation in them Consequently we would prefer the somewhat more descriptive 'vessels lacking blood corpuscles' Hawkins, who termed these vessels avascular channels' (1967), presumably sought to describe a possible degenerative phenomenon of the actual vessel by this term Again since they are found in the normal animal they may not indicate a degenerative phenomenon rather be a normal finding

The stria vascularis (Smith 1957, Mag 1966) and less frequently the limbus ves (Cimino & Grisanti 1967, Voldrich 19 have been thought to be involved in en lymph production and/or absorption The f that of the spiral lamina vessels the lim vessels are the only ones facing endolym would seem to be further indirect evidence their possible role in endolymph transfer

The density of RBCs in the various ves ranged from compact (packed) to spaced (μm apart) as shown in Table I The RBC den sity for each vessel and vessels with lumen influenced by EC and/or PC is shown sche matically in Figs 9 and 10 (See also Figs 4 5 and 7) Most importantly it can be seen from these figures that in the normal guinea p one can expect the density of RBCs in vessel to vary with or without corresponding ECs or

le I *The density of red blood corpuscles* *chlear vessels*

vascularis

e

ating arterioles – scala vestibuli apically
ating arterioles – scala vestibuli basally
lecting venules – scala vestibuli
vessel at the vestibular membrane
vessel of the spiral prominence
lecting venules – scala tympani
venule at the basilar membrane

se-Spaced

enous anastomoses
ibus vessels
vessel of the basilar membrane
vessel of the tympanic lip

ed

vessel of the scala vestibuli

's affecting the vessel lumen As previously ntioned, some authors (Lawrence et al, 7, Lipscomb & Roettger, 1973) found a de- n RBCs in the vessels below the basi membrane following noise exposure The sence of swollen endothelial cells affecting ssel diameter in the basilar membrane ves ls (Hawkins, 1971, Lipscomb & Roettger, 73) and in strial vessels (Lawrence, 1972) also reported in noise damaged ears As in Fig 9, in the normal guinea pig the of RBCs in the vessel of the basilar a ranges from close to widely- As is also seen, ECs can sometimes luence the vessel lumen On the contrary, g 10 shows that RBCs in strial vessels are ually quite compact ECs and PCs are diffi- ult to observe in the stria vascularis but seen do not seem to affect the dia- of the capillaries of the stria bed These eliminary findings suggest that the presence widely spaced RBCs in conjunction with wollen ECs partially occluding the vessel n in the vessel of the basilar membrane ollowing noise exposure cannot be viewed pathological finding since they are present normal animals as well Similar changes in he stria vascularis of noise exposed animals are more likely to be pathological, since they were not found in our control material In all probability, vascular pathology, regardless of cause, will not be manifested by qualitative differences between animals but rather by quantitative differences This fact, however, can only be determined by further in-depth investigations

We have previously noted a simplification in the vasculature apically (Axelsson 1968) In this study perivascular spaces in the spiral lamina vessels were found to be approximately twice as frequent in the two basal as in the two apical turns In addition the existence of granula surrounding the vessel of the basilar membrane was more prevalent in the three basal turns than in the apex The granula, which could be a by product of high ATPase activity, and the prevalence of perivascular spaces may be related to the previously demonstrated high metabolic rate in the cochlear base (Nakai & Hilding, 1967) Interestingly, in the external wall vessels, perivascular spaces were distributed fairly evenly through out the cochlea Earlier the hypothesis was advanced that perivascular spaces (and less frequently vessels lacking blood corpuscles) may be involved in the processes of perilymph transfer and absorption The differences in the distribution of perivascular spaces would seem to suggest that this function could be better carried out basally than apically, at least in the spiral lamina

In summary it should be restated that the vascular conditions described in this paper are for the guinea pig Preliminary studies of the chinchilla suggest that differences can (and do) occur between species We are reluctant to speculate, for example on the significance of the blebs seen in Fig 2 in the stria vascularis, since similar phenomena have not been observed in the chinchilla It is suggested, therefore, that these initial results be regarded with caution and that they should be substantiated, if possible, with further quantitative investigations of a similar nature

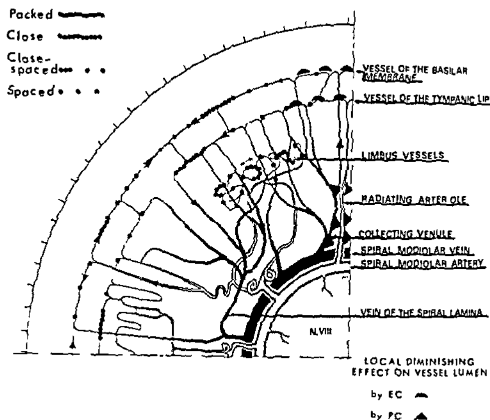


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COCHLEAR PATHOLOGY FOLLOWING EXPOSURE TO MERCURY

M Anniko and L Sarkady[1]

From the Department of Otolaryngology Karolinska sjukhuset and King Gustaf V Research Institute Karolinska Institutet Stockholm Sweden

(Received March 17 1977)

bstract The sensory and secretory epithelia may be ome morphologically changed following the exposure to

cytocochleogram studies) Acute intoxication mostly af ected both afferent and efferent nerve terminals and the air cells while chronic poisoning could also damage he stria vascularis

Since the classic paper published by Hunter t al in 1940 on mercury intoxication, many investigators (Takeuchi, 1970, Lofroth, 1969, Kurland et al 1960) have described the toxicology of mercury compounds There have been reports from all parts of the world concerning poisonings and even deaths resulting from the ingestion or inhalation of mercurials or from cutaneous contact with these compounds (Troen et al , 1951, Jalili & Abbasi 1961 Friberg 1971, Rosen et al , 1966) Comparatively few reports of neuro-otological observations following intoxication by mercurials (Nosaka et al , 1970, Fujisaki et al 1971) have been published describing dysequilibrium and impairment of hearing and speech discrimination as the main findings

According to the observations of Mizukoski et al (1975) the neuro-otological disturbances following chronic mercury intoxication may be considered mainly as lesions of the retrocochlea and of the oculomotor system in the brain stem and the cerebellum

To our knowledge the only animal experimental study on the effect of mercury on the inner ear was performed by Falk et al (1974) who described the degeneration of outer hair cells of the organ of Corti mainly at 2½ turns from the base of the guinea pig cochlea

The aim of the present study was to investigate morphologically the effect of inorganic mercury on the inner ear structures The present paper deals with the cochlear part of the labyrinth and the effects on the vestibular part of the inner ear will be evaluated in a separate paper

MATERIALS AND METHODS

Sixty two young healthy guinea pigs (250–350 g) were treated with daily subcutaneous injections of mercury chloride ($HgCl_2$ as a 0 5% solution in sterile water) in doses ranging from 2 5 to 25 mg/kg b w

The total dose ranged from 10 to 90 mg/kg b w administered during 1–49 days The survival time after the last injection varied from

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[1] Then visiting scientist at the Department of Otolaryngology Karolinska sjukhuset and King Gustaf V Research Institute Present address Department of Otolaryngology Semmelweis University Clinic Budapest Hungary

1 day to 1 month The control group of animals consisted of 9 young untreated guinea pigs

The animals were weighed daily and examined for general appearance and activity The gross neurologic investigation include gait disturbance, hindlimb crossing phenomenon and the presence/absence of Preyer's reflex

Morphological procedures

The cochlea was investigated by light and electron microscopy for both qualitative and quantitative analysis of cochlear damage Specimens were taken according to the technique of Wersäll (1956) The cochlea was perfused with 2% buffered (veronal acetate) osmium tetroxide solution and remained in the fixation fluid for 2 hours The specimen was dehydrated in alcohol and embedded in Epon

1 μm thick sections were taken for light microscopy after staining with toluidine blue and thin sections were prepared for electron microscopy (stained with uranyl acetate and lead citrate)

The quantitative analysis of the sensory cells in the organ of Corti was performed by phase contrast examination of surface preparation counting present and absent sensory ells a technique described by Kohonen, 65, Ernstsson, 1972, Ylikoski et al , 1973, Anniko, 1976

RESULTS

Clinical Findings

The clinical signs were divided into those following acute (10–25 mg/kg b w on each occasion) or subacute intoxication (7 5 mg/kg b w on each occasion) and those caused by chronic intoxication (2 5–5 0 mg/kg b w daily for several weeks)

The former group showed general weakness as the main symptom often combined with a loss of Preyer's reflex shortly after the injection The weight loss was minimal less than 5% of the initial b w Neurological signs did not appear The animals never showed a disturbance of the balance or of the righting reflex

The chronically treated group of guinea pigs showed great changes in their body weight Sometimes the weight decrease reached 45% of the initial weight However, an individual tolerance to mercury chloride treatment was obvious, and some animals increased in weight during this period of time

Many guinea pigs lost their Preyer's reflex which, however, sometimes returned after a few days or a week Crossing of the hindlimbs or ataxia was not observed

Morphological Investigation

I *The cytocochleogram*

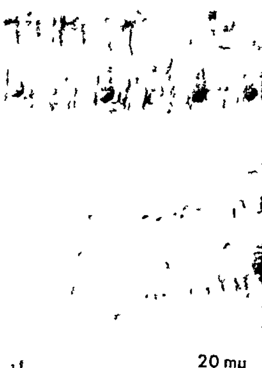
Normal distribution of cochlear hair cells in the 4th coil Missing outer hair cells (OHC) and an irregularity of the outer hair cell rows may occur in the organ of Corti in the 4th coil especially within 0 5 mm from the cochlear apex A complete cytocochleogram of the coil was made in the control group of guinea pigs Among the three rows of outer hair cells at 18 5–19 0 mm from the round window the third row of sensory cells showed 5–1 loss, the second and the innermost hair rows showed about a 15–20% loss sensory hair bundles of the inner hair cells (IHC) were missing in about 20% of the sensory cells

Fig 1 Interference contrast microscopy Chronic mercury chloride intoxication Degenerated outer hair cells (OHC) in the second (*OHC II*) and the third (*OHC III*) rows are replaced by phalangeal scarring Inner hair cells (*IHC*) are completely preserved

Fig 2 Light microscopy (LM) Chronic poisoning Degeneration of outer hair cells in the second and third rows (*arrows*)

Fig 3 Electron microscopy (EM) Damage to hair cell mitochondria (A) Chronic intoxication Increased electron density of the mitochondrial matrix and crista disintegration (B) Acute intoxication Herniation of the mitochondrial membrane Small intramitochondrial inclusion bodies adjacent to the cristae are frequently observed (*arrows*)

Fig 4 EM Chronic mercury chloride intoxication Degenerating nerve ending of the myelin figure type below outer hair cell (*OHC*)


20 mμ
1


50 mμ
2


1 mμ
0.5 mμ


OHC
1 mμ
4

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wl›

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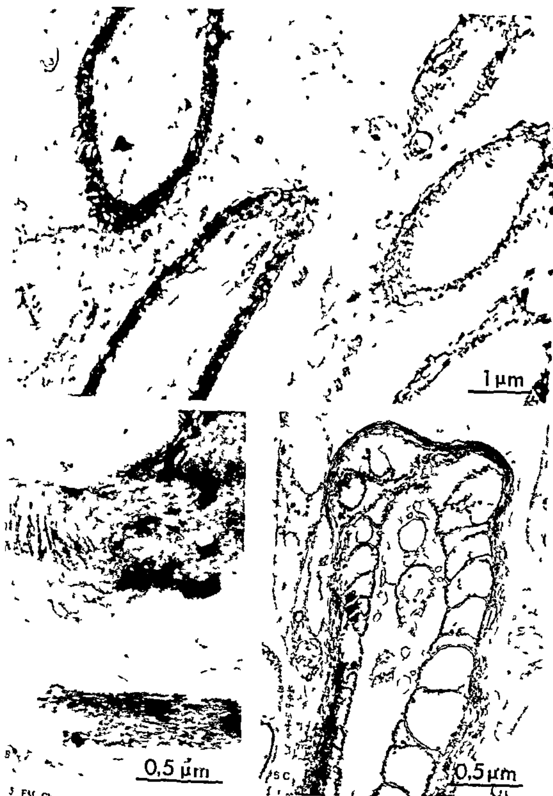
Chron› r ha› l (OH› er ha›

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Fig 4 EM Chronic mercury chloride intoxicatio› generating nerve ending of the myelin figure type be› outer hair cell (OHC)



5 EM Chronic intoxicat on (A) Myelinated nerve disintegrat ng (B C) Details from degenerat ng myelin sheaths showing d ssolut on of the myel n sheaths The axoplasm is lost and the myel n sheaths are

78°959

outer and inner hair cells showed a vesicular degeneration indicating a final, unspecific, common path of cell disintegration

Concerning the morphological changes, no difference occurred between afferent and efferent nerve terminals following acute intoxication, thus indicating that both types of neural tissue were affected at the same time If nerve endings appeared morphologically altered they were initially more affected than the adjacent hair cell although some cytoplasmic changes also occurred in the hair cell Ultrastructural changes of nerve endings did not occur in all investigated specimens, thus indicating a great variation in the susceptibility to mercury chloride poisoning

Degenerated nerve endings of the myelin figure type were observed below the hair cells following chronic intoxication, while the adjacent hair cell appeared ultrastructurally normal or had only minimal signs of cytological damage (Fig 4) Adjacent nerve endings were mostly undamaged, which may be explained by the long time elapsing from the last injection until the sacrifice of the animal, indicating that damaged nerve terminals already had been degenerated In the disintegrating efferent nerve endings the transmitter vesicles ere preserved even when the mitochondria ithin the nerve endings seemed greatly damaged

Morphological signs of demyelinization appeared in myelinated cochlear nerves following chronic mercury intoxication However, not all myelinated nerves were affected simultaneously, so many of them still appeared ultrastructurally rather undamaged while others were severely degenerating In the disintegrating nerves the normal compact sheath of myelin layers, tightly compressed together so the extracellular spaces between the layers are completely obliterated (multilayers of Schwann cell membrane) showed vesicular dissolution by an organized arrangement of equal-sized spheres (Fig 5 C) derived from the myelin lamellae of a damaged myelin sheath (intramyelinic split) but it could also present

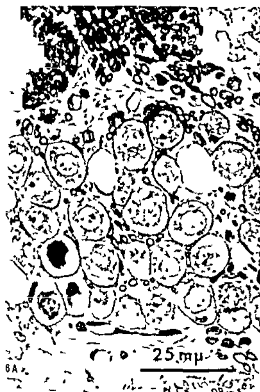
organelles) but in many degenerating elinated nerves foci of floccular dense bo were observed The cochlear ganglion were mostly preserved though scattered lo of ganglion cells appeared (Fig 6 A) Ac intoxication primarily affected the coch ganglion and sometimes caused a rapid integration of ganglion cells (Fig 6B)

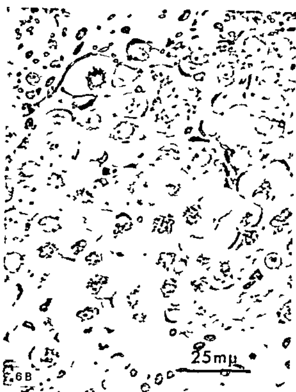
In most investigated specimens the vascularis appeared morphologically int However, an increased vacuolization of marginal cells could sometimes be obse following both chronic and acute poiso (Fig 9) In the evolution of damage to the vascularis, which was found mainly follo chronic poisoning, an intercellular oedem the intermediate cell layer occurred (Fig 10) but without affecting the basal cells intercellular oedema can reach a consider size but no epithelial cells were rejected the surface of the stria vascularis or found floating above it The mitochon changes became primarily apparent in th termediate (chromophobe) cells and the ginal (chromophil) cell extensions The phological transformation of their ultras ture following the two main types of ex mental conditions was similar to that ea described concerning mitochondria in cells (Fig 7 A, 7 B)

At the same level in the cochlea as the u structurally altered stria vascularis the sen cells of the organ of Corti were still pre but appeared often distorted with an incre

Fig 6 LM Cochlear ganglion cells (A) Chron toxication Most ganglion cells are preserved and scattered losses of cells appear (B) Acute intoxicat general disintegration of the ganglion cells

Fig 7 EM Morphological transformation of mito dria in marginal and intermediate cell extension Acute intoxication Swelling of mitochondria with disintegration (B) Chronic poisoning Distinct inc in electron density of the mitochondria so that the c cannot be identified


25 mµ
6A


25mµ
6B


0.5 mµ
7A


0.5 mµ
7B

density of the cytoplasm and some degenerating nerve endings below them However, morphologically similar hair cell changes were also observed without corresponding alterations of the stria vascularis The supporting tissues of the organ of Corti appeared unaffected by the mercurial poisoning

DISCUSSION

Recent findings of high levels of mercury in the environment have raised questions concerning its effects, and the site and mode of cellular action The clinical features of mercurial intoxication are well known as the Hunter Russel syndrome, and the pathological findings at autopsies of such cases showed that *the neurological disturbance when investigated* a long time after the intoxication was attributable to the destruction of neurons in the central nervous system (Hunter & Russel, 1954, Takeuchi et al, 1962, Oyake et al, 1966)

Neuro-otological studies of intoxication by mercury have shown that the hearing impairment in the early or middle stage of the intoxication may be caused by cochlear lesions but at a later stage mainly result from retro cochlear lesions (Mizukoski et al, 1975) Kurland et al (1960) reported from the Minamata disease that *hearing impairment (sometimes* even a total loss) was an early and frequent finding following mercury poisoning (observed in more than 80% of all patients)

In the present study the degree of hearing was estimated by means of Preyer's reflex, which rapidly disappeared following acute intoxication This was, after ultrastructural investigation of the specimens, interpreted *mainly as an effect on the neural structures* The sensory cells were surprisingly well preserved although sometimes minor cytological damage occurred while the adjacent nerve endings (both afferent and efferent) were more seriously affected In the initial stage of intoxication, these changes are likely to be reversible Preyer's reflex often returned and when the animals were later investigated ultra structurally the damage was less pronounce[d] than when studied immediately after the i[n]toxication In chronic mercury poisoning Preyer's reflex did not disappear until late du[r]ing the treatment and this was likely due not [to] neural damage alone, as degenerating ha[ir] cells occurred more frequently than followi[ng] acute poisoning

Reports have been published indicating th[at] the inner ear might have a delayed capaci[ty] to eliminate certain toxic substances thereb[y] exposing the cochlea to a high concentrati[on] *during a long period (Stupp, 1970, Stupp et a[l]* 1973, Anniko & Plantin, 1977) Falk et a[l] (1974) revealed that there is a slow, contin[u]ous increase in mercury concentration [in] blood during chronic intoxication Thus e[x]posure of the inner ear to mercury would i[n]crease

Toxic effects following metallic and [in]organic mercury poisoning are produced af[ter] oxidation of the elemental mercury to m[er]curic ions in vivo (Kubasik & Volosin 19[7..] Ulfvarsson (1969) reported that the distri[bu]tion of mercury among the organs was [in]dependent of the mode *of administration*

Degeneration of myelinated nerves is a l[ate] mercury effect while the damage to nerve t[er]minals occurred early during the intoxicati[on] *The early predilection* for the peripheral n[er]vous system is consistent also with other [re]ports (*Miyakawa et al*, 1970, 1971) Abnorm[al] myelin sheaths have also been shown by H[…]man et al (1973) in their ultrastructural st[udy] of mercury induced primary neuropathy in [the] rat

Mitochondria in hair cells and nerve t[er]minals were the first structures to become [af]fected This *indicates also* changes in th[e]

Fig 8 EM Chronic intoxication Stria vascularis [...]sicular degeneration of a marginal cell extension adja[cent] *to a strial blood vessel (BV)*

Fig 9 EM Acute intoxication Stria vascularis [in]creased vacuolization of marginal cells (*MC*)

Fig 10 EM Chronic intoxication Intercellular oed[ema] of the stria vascularis The basal cells (*BC*) appear [un]affected *MC* marginal cells


8
2 µm


MG
MG
2 µm

MG
MG
BC
BC
10
5 µm

functional state Swelling of the mitochondria is regarded to be an early sign of degeneration caused by a change in the ion balance across the outer mitochondrial membrane Swelling is often a reversible state and has been observed in mitochondria after treatment with various drugs (Kanda & Igarashi, 1969) Southard et al (1974) reported that mercurials and other heavy metals at low concentrations profound ly affect the control of the mitochondrial function Oxidative phosphorylation, calcium transport and phosphate accumulation are suppressed, whereas ATP hydrolysis, K^+ transport, Mg^{2+} transport and mitochondrial configurational change are induced As mitochondrial respiration diminishes, there is a simultaneous loss of cristae mitochondriales and a change in membrane permeability producing swelling is a common feature (Howell et al , 1971, Hwang et al , 1974) The reason for rupture or herniation of mitochondria is difficult to state It may be a consequence of the swelling process or due to an increased susceptibility of the membrane following exposure to mercury to the fixation procedure, thus producing these herniations (Bagger-Sjoback & Wersall, 1977)

The appearance of mitochondria with an ex- , dense amorphous matrix without visible cristae following chronic mercury chloride intoxication may be an effect of a prolonged compensated leakage of ions due to a mercury induced disturbed non lethal membrane permeability but a direct degenerative transformation of mitochondrial lipo proteins cannot be excluded The inhibition of cell respiration and ATP synthesis by Hg^{2+} suggest a possible explanation of the toxi cological properties

The effect of chronic mercury poisoning in the inner ear of the guinea pig has been ex amined histochemically by v Westernhagen (1969) who reported metabolic disturbances in both the sensory cells of the organ of Corti and the stria vascularis, without morphologi cal changes He concluded that hearing dis orders following mercury intoxication are at least in part, likely to be caused by the block age of important metabolic pathways befo other signs of cellular damage appear

Nakazawa et al (1975) observed that the ef fect of mercury compounds was due to an in hibition of DNA synthesis thereby damagi the machinery of macromolecular synthes Similar results were obtained by Li & Trach (1974)

In the location of its damaging effect me cury is similar to atoxyl causing degenerati of the sensory epithelium of the apical coi (Anniko, 1976) in contrast to most oth known causes of cochlear hair cell dama (Ylikoski et al , 1974) There is however discrepancy between the location of the pr ent pathological findings to the apical part the cochlea and the high frequency deafne often described in mercury intoxica humans (Falk et al , 1974) Damage to t guinea pig cochlea in the 3rd and 4th co l likely to produce a low frequency hearing l below 2 000 Hz (Ylikoski et al , 1973)

cochlea) may early be damaged during cury intoxication Hearing impairment wo therefore not only depend on the mercu effects on the central neuronal pathways l also be related to morphological changes in t inner ear

ACKNOWLEDGEMENT

The authors wish to thank Professor Jan Wersäll Dep ment of Otolaryngology Karolinska sjukhuset for interest in this work and for his critical revision of

fully acknowledged

ZUSAMMENFASSUNG

Die ersten und größten Veranderungen der sensorisc Ep thel zeigten sich in dem ap kalen Teil der Coch wahrend der basale Teil nur selten beschäd gt war (C cochleogramm Studien) Die ultrastrukturelle Un suchung erwies daß sowohl d e sensorische wie d e sel torische Epithel durch Merkurium Chlorid beschä werden können Akute Intoxikation griff meist d e N

.. (afferente sowie efferente Nervterminale) an wo- ... chronische Vergiftung auch andere Strukturen Cochlea beschadigt

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HEARING IN POP MUSICIANS

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bstract Comparatively few previous studies have in stigated the hearing of pop musicians On the average surprisingly low percentage (5%) of hearing loss was emonstrated in 160 pop musicians The present material omprises 83 musicians with an average exposure of 9 ears Group mean hearing thresholds show only a slight eviation from normal Age weekly and total exposure me appear to increase the risk of hearing impairment n the individual analysis 13–30% were found to have a ensorineural hearing loss depending upon the defini on of hearing loss Subjects with hearing loss show a screte impairment in the frequency range 3–8 kHz onsidering the sound levels and the length of exposure he incidence of hearing loss is surprisingly low Pos ble reasons for this are discussed

Vhat the younger generation considers to be heir legitimate right is often in pronounced opposition to the morals or tastes of the older eneration Nowadays, the performance of dance music constitutes such a problem The younger generation wants to hear their pop music electronically amplified to very high sound levels, both when dancing and at con certs This not only irritates the older genera tion who are quite often subjected to "noise pollution at home or at work, but also awakens the parents' sincere concern for the hearing of their children One of the reasons to suspect that high volume pop music leads to a sensorineural hearing loss (SNHL) is the subjective tinnitus and temporary threshold shifts (TTS) experienced by musicians and their audiences after exposure to one or more of their performances The relationship be tween a temporary and a permanent hearing loss, however, has not been conclusively elucidated Nevertheless, it is quite apparent that those performing the music are those who are probably most heavily exposed to the high sound levels Consequently, pop musicians should constitute the group where SNHL should be found, if pop music does, in fact, cause such hearing loss In relation to the substantial number of general publications centering around these questions, surprisingly few investigations have concerned themselves with possible permanent hearing loss in pop musicians The hitherto published investiga tions analysing hearing individually are pre sented in Table I A compilation of these cases shows that a permanent hearing loss was found in 5% of the pop musicians, irrespective of exposure time, age, etc In contrast, using group mean analysis of hearing Fearn (1973) demonstrated a statistically significant SNHL in the frequency range 1–6 kHz in attenders at pop music performances when compared with non attenders, 1 4–3 3 dB Attenders showed increased hearing loss with increased attend ance but also with exposure to gunfire Flet cher (1973) showed a sensorineural hearing loss at very high frequencies (≥8 kHz) in young pop musicians, 18–21 years of age Red dell & Lebo (1972), also adopting group mean analysis of hearing, showed a sensorineural dip at 6 kHz in 43 pop musicians with a mean age of 22 years

The aim of the present investigation was to examine the hearing in pop musicians

Table I *Pop musicians Frequency of permanent hearing loss (PHL) caused by pop music*

Author	Number examined	Number PHL
Fluur (1967)	13	0
Rintelmann & Borus (1968)	42	2
Ewertsen (1971)	26	0
Jatho & Hellman (1972)	65	5
Chuden & Strauss (1974)	14	1
	160	8 (5%)

MATERIAL AND METHOD

By advertisements in the news media and through personal contacts, pop musicians were asked to attend the examination Tested musicians were asked to encourage their colleagues of the band and other musicians they knew to take part in the examination In many cases it was possible to examine all members of a pop band at a training session or concert In these instances, hearing was tested in a mobile van A total of 69 musicians, 4 disc jockeys, 4 managers and 6 sound engineers were included in the audiological examination Of the total 83 tested, 52 constituted all the members from 8 pop bands, while the rest were members of many different pop bands This material includes 18 musicians from three internationally well known English pop bands The individual distribution with respect to the type of musical instruments played is presented below

guitar	26
base	8
piano or electric organ	12
drums	16
saxophone	4
singers	4
violin	1
more than one instrument managers sound engineers etc	12
	83

The average age of the group was 26 5 years (17–40 years) The average exposure time for the musicians was 9 3 years they performed 18 3 hours (average) a week 3 hours each session

Pure tone air conduction thresholds were obtained at both ears for all musicians When the thresholds exceeded 20 dB, bone conduction testing was also carried out Pure tone audiometry was performed in a sound proof booth The audiometers used were Madsen TBN-80, Madsen OB 70 and Kamplex Al data were computer analysed

Statistical treatment was performed according to Z-test, using a significance level [illegible]

Hearing loss is here defined as a mean threshold level greater than 20 dB at one of the frequencies 3–8 kHz at one ear

RESULTS

Group means

The average hearing in pop musicians is presented in Fig 1 It can be seen that there is slight difference between the ears, in that the right ear is better in the low frequencies and the left ear is better in the high frequencies This is due exclusively to a marked difference between right and left ears in younger musicians It can also be seen that thresholds are most severely impaired at 6 kHz with an average of 20 4 dB at the right ear and 17 1 dB at the left ear

The material was analysed in many ways in order to establish parameters contributing to possible SNHL The results are presented elsewhere (Axelsson & Lindgren 1977)

In summary, the following parameters

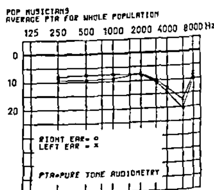
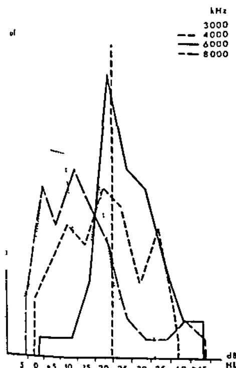


Fig 1 Average pure tone audiogram



2 Histogram showing pure tone loss in relation to equency

ear to have a negative influence on hearing in op musicians

lder age
ong exposure time
ng weekly exposure
exposure per session
laying drums
icipation in military service
stening with stereophonic headphones

vidual analysis

In the individual analysis each ear with a pure threshold exceeding 20 dB at any fre ency from 3 to 8 kHz was taken into ac- It was found that 38 musicians had a HL The right ear only was affected in 11 the left only in 5 cases, and both ears in cases

A total of 13 cases were excluded as their was reasonably explained by causes ther than pop music These causes were

congenital loss	1 case
age (according to Spoor 1967)	5 cases
SNHL unknown etiology verified before exposure to pop music	3 cases
Occupational hearing loss (other than from pop music)	4 cases
Total	13 cases

After exclusion of the 13 cases 25 pop musicians remain with SNHL, i e thresholds exceeding 20 dB at least at one frequency from 3–8 kHz at one ear

Contrarily, certain other possible causes of SNHL were not regarded as sufficient to exclude the patients from the material These were

military service (4–19 months)	9 cases
previous otitis media	7 cases
bilateral mastoidectomy	1 case
amateur shooting	2 cases
head concussion	1 case

The distribution of the hearing loss at the high frequencies for the 25 musicians is shown in Fig 2 It can be seen that most ears are within normal limits and that there is a considerable difference between frequencies At 3 and 8 kHz few ears were poorer than 25 dB The frequency 4 kHz shows a fairly equal distribution of ears above or below this level, while many pathological ears are found at 6 kHz, which is the most commonly impaired frequency It can also be seen that very few ears exceeded a 35 dB hearing level

To establish the incidence of SNHL, three different limits were set, as shown in Table II It can be seen that the SNHL in pop musicians in the present material ranges between 13 and

Table II *Incidence of sensorineural hearing loss*

No tested	83
No with SNHL>20 dB in one ear and one frequency (3 4 6 8000 Hz)	38
No with SNHL after correction for age	
	25 (30%)
	18 (22%)
	11 (13%)
	Unilateral 7 cases (8%)
	bilateral 4 cases (5%)

Table III *The worst cases (pure tone audiometry >20 dB in one or both ears)*

Subj no	No tested musicians in the band	Age	Instrument	Played years	Plays (hours/ week)	Plays (hours/ session)	Milit service (mo)	Otitis media	Head concussion	Mean hearing loss (in dB 3 4 6 8 kHz) Right	Left	Max hearing loss in dB (frequency kHz) Right	Left
1		28	Guitar bass	8	50	2	4	+		24	11	45 (6)	20 (6
2		40	Guitar	18	18	3	12			26	30	40 (8)	55 (8)
3	5	35	Piano organ	16	25	4	–	+		25	13	35 (4)	25 (4)
4	7 Same band	32	Bass trombone	10	10	1	10			21	23	25 (?3)	30 (4)
5	7 Same band	35	Singer	20	10	1	19		+	23	25	30 (4)	25 (6)
6	6	28	Bass guitar	16	30	4	13			11	25	20 (4 6)	40 (4
7	5	27	Stage monitorist	5	70	1 5	–			14	29	20 (4)	40 (8
8	6 Same band	27	Bass	9	30	2	–			29	31	40 (6)	35 (4
9	6 Same band	28	Drums	15	50	2	–	+		18	23	30 (6)	35 (4
10	6 Same band	29	Singer	10	20	2	–	+		20	24	25 (6)	30 (6
11	6 Same band	28	Guitar	15	50	2	–	+		21	20	35 (4)	30 (
Total average		30 6		13	33	2 2							
Total material average		26 5		9 3	18	3							

30%, depending upon the definition of hearing loss In most cases the SNHL was discrete The 11 worst cases of SNHL in pop musicians are presented in Table III The cases had an average loss from 3–8 kHz greater than 20 dB It can be seen that these cases were, on average, 4 years older than the group as a whole In addition they had played an average of 13 years, compared with the overall average of 9 3 years The weekly exposure for these musicians was 33 hours and they had played an average of 2 2 hours/session, the means for the whole group were 18 hours and 3 hours, respectively The table also shows the average hearing in the high frequencies and this again confirms that the hearing loss was, in general, small

DISCUSSION

Occupational noise is often considered more or less "necessary" and difficult to avoid In contrast, noise during leisure activities seems more "unnecessary" and easier to avoid It also appears particularly important for th exposed to occupational noise to avoid no leisure activities, in recent years a new, no leisure activity has been introduced in society, i e amplified pop music As a sign the continuous protest of the young generat toward "old folks" or for other unkno reasons this pop music is presented at h sound levels, clearly above the hearing d age risk level Occasional sound level me urements as well as noise dosimetry have b used as "proof" of the expected noxious fect of this music on hearing Screening te have shown more young individuals to hav hearing loss, which has also been blamed pop music (Lipscomb, 1969*a*) Howev amazingly few investigations have reported the individual hearing in those obviously m exposed to the pop music, i e pop musicia Those studies that have been done h demonstrated a surprisingly low incidence SNHL (Table I)

The following factors have been conside as evidence of the harmful effect of pop mu on hearing

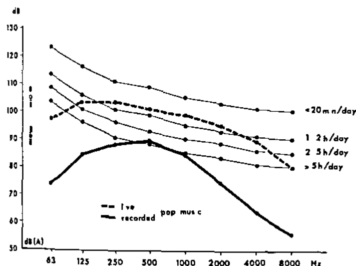


Fig 3 Live and recorded pop music in relation to the Swedish damage risk criteria

ry high sound levels
gh equivalent sound levels
TS after exposure
earing screening showing young individuals with hearing loss
idely exceeded established damage risk criteria
npaired hearing in pop music attenders in comparison with non attenders in hearing tests (Fearn, 1973)
air cell damage in inner ears subjected to pop music, found at histological examination (Lipscomb, 1969*b* Bohne et al , 1976)

How is it then possible that previous studies ound only 5% of pop musicians to have NHL and the present investigation found 3 or 30%, depending upon where the limits or normal hearing are set? Certain factors end to explain such a low occurrence of earing loss The dominant frequencies of op music are low, the 250–500 Hz range is naximally amplified Low-frequency noise is ss damaging to the inner ear, perhaps hrough the protective action of the stapedius eflex The presentation of loud pop music is often interrupted by pauses which offer at east some possibility of recovery and rest The exposure time is very different from that in industrial noise exposure For listeners this would probably amount to 2 nights/week (6–8 hours) and for pop musicians probably 2–3 times that, such as the 18 hours average found in the present investigation

Lately, an increasing amount of evidence has accumulated demonstrating the relatively high risk of hearing damage from impulse noise in relation to other types of noise (Bruel, 1976) Pop music is presented amplified over loudspeakers and is characteristically a fluc tuant noise with little inclusion of impulses These factors tend to make the picture of hearing loss in connection with pop music some what less disastrous Two other possibilities to explain the low incidence of SNHL in pop musicians have been suggested by Fearn (1973) He believes that those pop musicians experiencing a substantial hearing loss, either temporary or permanent possibly quit playing earlier than their less sensitive companions It is also possible that those musicians with a permanent hearing loss avoid taking part in this type of examination On the other hand, reservations also have to be made that hearing testing was performed on only one occasion, which would tend to give inferior results rather than the reverse Also in some instances the testing was performed immediately before a concert and there is no certainty that the musi- cians did not have some TTS, e g

posure to sound tests on stage before the concert

Whittle & Robinson (1974) presented a compilation of sound levels *from many* different *investigations* If these average sound levels are related to the Swedish noise damage risk criteria it can be seen that recorded *music in* discotheques on average *hardly* reaches hearing damage levels if exposure *time is less than* 5 hours (91 dB(A)), Fig 3 In this *connection it* is important to emphasize *the possible* harmful *effect of* listening to pop music with *stereo* headphones such that *much higher* sound levels are incident on the drum *membrane In* Fig 3 we can also *see the average* octave analysis of live pop music It can be *seen that an* unprotected ear could *be exposed* approximately 30 min/day to live *pop music* (*105* dB(A)) without risk *of* hearing loss If *this* figure is converted to a weekly *exposure of* 2 5 hours it appears that occasional *patrons of* discotheques *could expose* themselves to the live pop *music once* or twice a week *without* risk of hearing loss It is *obvious that* the picture is clearly *different for* pop musicians In general, pop musicians are exposed to the loud music for *many more* years than the audience It is also clearly seen that *pop musicians with* average exposure *of 18 hours* a week, as in the *present investigation*, far exceed the risk level *In this* respect the finding of a *discrete* SNHL in 13–30% of *pop musicians* which seldom *exceeded 30* dB at any frequency, is surprising *The* group mean hearing, though, shows *a dip* at 6 kHz, very similar to that *presented* by Reddell & Lebo *(1972) and* indicative of an early *slight* sensorineural impairment in these *fairly young* individuals

There is a well known *large* inter individual *difference* in sensitivity to noise *Basically, we* do not know what factors *contribute* to these inter-individual *differences* We would like to *make the suggestion* that there may be a difference in inner ear circulation on a hormonal *basis if high* sound levels are experienced as "beautiful music" versus as terrible *noise*"

The male ear, *particularly is* exposed to *many different* noises from early childhoo squeaking toys fire crackers, toy guns g *fire* in military service, occupational nois etc *It is obvious that* in general no single fa *tor* can be blamed for an individual hear loss but that such a loss rather is the result repeated trauma to the inner ear *Consequen ly, there is* seldom reason to incriminate p music *per se* as the cause of hearing loss Co *trarily*, it is important to remember that a n mal *audiogram or one with* only a slight he *ing* loss does not necessarily reflect t amount of morphological damage to the inn ear Even with a normal pure tone audiogra *sensory cell* loss can be substantial Furth more, the presentation of pop music at th high sound levels appears senseless If t younger generation considers high sou *levels* necessary for enjoyment of their mu it is our obligation to determine the high acceptable sound level for these perf mances A level of 105 dB(A) is unaccepta *from* the audiological point of view A level 85 dB(A), the Swedish hearing damage r level, is probably unacceptably low from audience point of view Maybe a level of proximately *95 dB(A)* would satisfy both p ties in being noisy enough to be enjoyable the one hand and restrained enough to p serve normal hearing on the other

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ZUSAMMENFASSUNG

Es gibt verhaltnismaßig wenige Studien uber das G der Popmusiker Überraschend wenige Musiker mit verlust wurden gefunden im Durchschnitt 5% von Popmusikern Unser Material enthält 83 Popmusiker einer durchschnittlichen Exposition von 9 Jahren Gehör der Musiker zeigt im Durchschnitt nur e geringen Verlust Der Risiko eines Gehorverlustes s mit Alter Expositionszeit pro Woche und Gesamter tion In der individuellen Analyse wurde ein Gehörve in 13–30% gefunden abhangig davon wie man norm Gehor definiert Die Popmusiker mit Gehörverlu

ine diskrete Verminderung in 3–8 kHz Mit Hin auf die Schallpegel und die Expositionszeit ist die •nz von Gehorverlusten erstaunlich klein Ver lichkeiten dieses zu erklaren werden dis

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A CLINICAL PILOT STUDY ON PREFORMED AUTOLOGOUS OSSICLES II

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Abstract A research project concerning preformed autologous ossicles has been going on for more than ten years Animal experimental studies were followed by a clinical pilot study which showed that bone could be produced in a titanium mould placed in the proximal tibia metaphysis In the present investigation in which another five patients were operated on the experimental design was changed compared to the first pilot study and bone production was improved In addition to the tibial moulds ten titanium cylinders were placed around a bony bridge prepared in the linea temporalis of the ear to be reconstructed Nine out of ten of these cylinders contained bone suitable for ossiculoplasty Histology showed higher osteocyte density and a higher proportion of vital looking osteocytes in the preformed grafts compared with a peroperatively sculptured graft of cortical bone On histochemical investigation the preformed graft cells seemed viable at the time of transplantation The technique for obtaining a preformed autologous ossicle the titanium cylinders in the temporal bone was to perform and the patients did not experience any The risk of infection in this area was judged be small

A project concerning preformed autologous ossicles has been going on since the late nineteen sixties In a series of animal experiments it was shown that bone tissue with specific anatomy could be produced in titanium moulds placed in the proximal tibial metaphysis It was also shown that this bone could be produced in a suitable shape and size for use as an ossicular prosthesis This experimental work was published in 1976 (Hallén et al , 1976)

The first clinical pilot study on preformed autologous ossicles started in 1976 and was recently reported (Tjellstrom et al 1977) Titanium moulds with room for two ossicles each were placed in the proximal tibial metaphysis of 5 patients with ossicular defects When the moulds were extracted bone was found in seven of the chambers but only in two of them was the bone stable enough to be used as a prosthesis

Based on the results of the first clinical pilot study, the next phase of the project was planned and carried out during 1976–77 The results are presented in this report

The aims of clinical pilot study II were

1 to find out whether the quality of the bone used for ossiculoplasty could be improved as compared with pilot study I The following changes were decided upon

(*a*) to put bone tissue into the chamber of the mould at the time of insertion

(*b*) to redesign the chambers, making the narrowest parts slightly wider

(*c*) to change the level of the mould in relation to the periosteum and the cortical endosteum,

(*d*) to shorten the time between insertion and removal of the moulds,

(*e*) to change the arrangement for fixation of the mould to the bone,

2 to find out whether preformed ossicles could be obtained more simply by implantation of a cylindrical titanium mould in the mastoid process of the ear to be reconstructed

3 to find out whether there were any differences concerning histology and histochem-

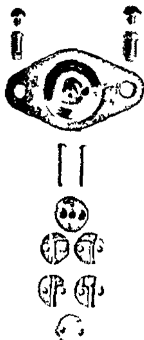




Fig 1 a The titanium mould assembled and taken apart

try between preformed ossicles and non-preformed ossicles prepared and shaped out of bone taken from the mastoid process

MATERIAL

The patients were selected from the waiting list for ear surgery at the ENT Department, Sahlgren s Hospital, Goteborg, Sweden, according to the same criteria as in the clinical pilot study I, viz

1 age between 20 and 50,
2 known or strongly suspected ossicular damage,
3 a dry ear without discharge for at least one year,
4 no signs of osteitis or cholesteatoma,
5 a good speech discrimination score,
6 normal hearing in the contralateral ear,
7 no general disease,
8 a normal radiological examination of the proximal tibial metaphysis

Table I *Preoperative situation in each of the patients (unfilled=defect)*

Patient	Sex	Age	Diseased ear	Drum	Ossicular defect	Pure tone audiogram	Speech dicr score	A/B gap
A A	♀	26	dx	atrofic but intact		55 dB	100%	42 dB
S K	♀	36	sin	thick but intact		43 dB	96%	28 dB
E L	♀	40	sin	normal		76 dB	64%*	55 dB
A N	♂	32	dx	normal		70 dB	76%	47 dB
H F	♂	24	sin	atrofic but intact		35 dB	96%	23 dB

*at 95 dB

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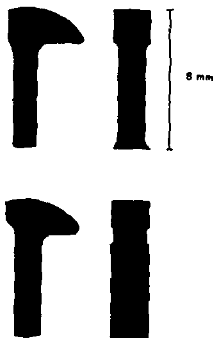


Fig 1b Shape and size of the chambers in the mould Upper figures intended for cases in which only the foot plate was left Lower figure intended for patients with an intact stapes

Five patients were selected and clinical data for each patient are presented in Table I

All the patients were thoroughly informed about the aims of the investigation and the potential risks with this type of surgery

For the histological and histochemical in-vestigation ten pieces of cortical bone w taken from the mastoid process of pati undergoing surgery for chronic ear disease

METHOD

(A) Five moulds were made out of titan Each mould contained two chambers a one for use if the stapes was intact and a row one in case only the stapedial foot was left in the middle ear (Fig 1) The rangement for fixation of the mould to bone was as shown in Fig 2 and a st arrangement was achieved with two sepa titanium screws

(B) Ten titanium cylinders were structed The shape and size of the two are shown in Fig 3 Each cylinder half two small grooves where a suture coul applied to adapt the pairs of cylinder h snugly to each other All inside surfa moulds and cylinders were carefully pol and then cleaned and autoclaved as in the first pilot study

Surgical procedure IA

The procedure described in pilot study I followed where applicable Anaesthesia given and the soft tissue was handled in

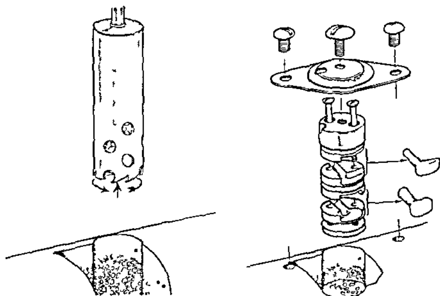


Fig 2 Extraction of bone plug and fixation arrange- ment of the titanium mould in tibia.




g 3a The titanium cylinders for the linea temporalis




Fig 3b Shape and size of the titanium cylinders

me way as in study I At the level of the berositas tibiae a hole was made in the nterior medial surface of the tibia with a ephine burr and a cylinder of bone was taken t From the cortical endosteal layer of the xtracted bone plug a slim bone prosthesis as prepared with a diamond burr The pre aration was done under irrigation with the pa tient s own plasma This bone prosthesis was placed in the wide chamber Care was taken to adjust the level of the chamber close to the periosteal layer but entirely within the bony cortex The narrow chamber was filled with spongy bone and red bone marrow, taken from the opened marrow cavity The mould was then put in place and immobilized by the

able II *Schematic illustration of the amount of bone produced in each patient (unfilled= efect)*

atient	Chamber close to the cortical endosteum	Chamber towards the marrow cavity	Preformed bone in os temporale
A A			
S K			
E L			
Å N			
H F			

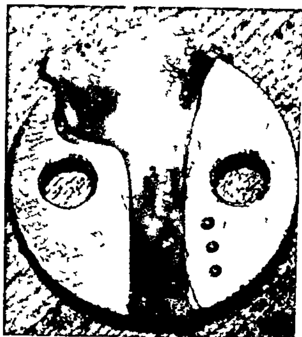




Fig 4 Preformed autologous ossicle from the tibia mould (*Upper*) The ossicle in the chamber half (*Lower*) The ossicle taken out ready to be used for the ossiculoplasty

fixation arrangement The level of the deepest of the chambers was close to the cortical endosteal layer

The periosteum and other soft tissu replaced The rest of the surgical pro and after care of the patient was the s described in the first pilot study

Surgical procedure I B

This procedure was performed at the session as procedure I A Under loc aesthesia with 5–10 cc of 1% Xylocain epinephrine 1 100000, an incision was behind the ear with the ossicular dam periosteal flap was elevated and the temporalis was identified Under ge saline irrigation to minimize heat traum bony bridges were prepared and two ti cylinder halves were adapted around The cylinder halves were kept in plac silk sutures placed in the groove on the o of the cylinder One short and one long der were used in each patient The peri flap was replaced and kept in place with catgut sutures and the skin was sutur bandage was applied but the next day th removed and only a piece of adhesive tap left in place The skin sutures were rem one week after surgery

Surgical procedure II

The second operation was planned and ca out according to the following schedule months after insertion of the moulds an cylinders the patient was admitted to hospital for the ossiculoplasty The recons tion was done under general anaesthesia the middle ear was exposed through a auricular incision The titanium mould taken out from the tibia and the cylinders exposed On removal of the cylinders cient bone was taken to permit sculpturing head for a prosthesis The bone that was cally judged likely to give the best result used for the reconstruction All handling o preformed bone was done in a glass ve containing the patient s own plasma. In tients with a perforated tympanic membr autologous temporal fascia was used for g ing with an underlay technique When sup



ig 5 Preformed autologous ossicle from the linea (*Upper*) One cylinder half taken away (*Low*) The ossicle taken out ready to be inserted into the ddle ear

drum graft was needed, Gelfoam® was ised No Gelfoam® was allowed to come into with the new ossicle The ear canal packed with rayon strips and acrylic cot- which was removed one week after the operation

Preformed bone from the tibia mould and os temporale cylinder of the 5 patients not d for the reconstruction were secured for and histochemical investigations all the cases it was possible to get a piece of e from the wide tibia mould for these investigations Cortical bone from the mastoid was taken from 2 of the patients and adjusted to the shape of an ossicular prosthesis with diamond burrs under generous saline cooling Another eight grafts of the same kind were taken from other patients and prepared in the same way in order to compare non preformed bone with preformed bone

All specimens for histology and histochemistry were placed directly in the operation room into Histocon (Histolab , Goteborg) and were within half an hour placed in 10% EDTA of pH 6 95, +4°C (Fullmer, 1966) (The histochemical investigations were performed by Professor Guy Heyden, Department of Histopathology, University of Goteborg) All preparations were decalcified in EDTA for ten days After decalcification the preparations were frozen in isopentane at −140°C The specimens were then sectioned in a cryostat at −20°C They were stored for complementary analyses at −70°C Preparations for histology were stained with hematoxylin eosin and van Gieson For lipid histochemistry oil red was used The enzyme histochemical methods included registrations of adenosine triphosphatase Ca ATPase pH 9 4 (Magnusson & Linde, 1974), $NADH_2$- and $NADPH_2$-diaphorase (Chayen et al , 1973), alkaline and acid phosphatase (Barka & Anderson, 1963) activities The enzyme histochemical tests were performed in order to disclose metabolically active cells ($NADH_2$- and $NADPH_2$-diaphorases) involved in hard tissue function (ATPase, alkaline phosphatase) and hard tissue resorption (acid phosphatase) The ATPase recordings could also be employed to show the distribution of viable blood vessels (Heyden, 1969), as defined by histochemical method

RESULTS

The clinical findings in each patient are presented separately below and a schematic illustration of the amount of bone found in each mould is presented in Table II

Patient A A Bone was found in the

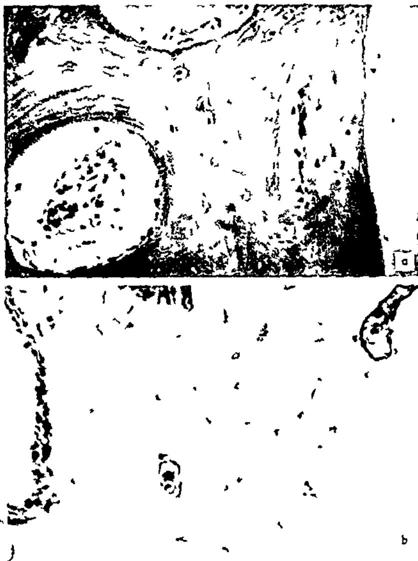


Fig 6 Preformed bone from the t bial mould of pat ent A A (a) Th lacunae contain v tal osteocytes throughout the preparat n (b) Histochemical investigat on show strong activity of NADH-enzym

chambers of the mould placed in the tibia Clinically, the bone tissue was stable enough to be used as a prosthesis (Fig 4) A thin coating of soft tissue was found around the ossicles In the titanium cylinders placed in the linea temporalis at surgical procedure IB clini cally stable bone was found and here too a coating was noted (Fig 5) This fibrous coat ing seemed to be thicker than that of the prosthesis obtained from the tibial mould For the reconstruction of the ossicular defect the wide prosthesis from the tibia mould was selected and an assembly technique was used

Patient S K The findings were as in pa tient A A and the reconstruction was performed with the wide tibial prosthesis from the stapes head to the drum (slippe prosthesis)

Patient E L The findings were as in A A and the same type of reconstruction wa performed

Patient Å N In the wide parts of th chambers of the tibia moulds bone tissue wa found but the shafts were not stable and co sisted only of fibrous tissue Both the cylinder from the linea temporalis, on the other han contained stable bone tissue with a fibrou coating and the shorter of these was used f the reconstruction using an assembly tech nique

Patient H F In the wider chamber of th tibia mould clinically stable bone tissue wa



Fig 7 Preformed bone from the os temporale cylinder of patient S K (*a*) The lacunae contain vital osteocytes A soft tissue layer lines the preparation (*b*) $NADH_2$-activity is strong in the bone and in the soft tissue layer

ind but the narrower one had only a fibrous t and could not be used as a prosthesis f the bony bridges prepared in the linea ralis at surgical procedure IB one had i resorbed at its most central part whereas other was well retained There was one ifference as compared with the other pa ents however The fibrous coating seemed be somewhat thicker in this case and it was iot firmly attached to the bone tissue The ide prosthesis from the tibia was found suit and was used for the reconstruction with malleus–stapes assembly

All patients in this study complained of discomfort from the leg but much less i in the previous study None of the patients complained of any discomfort from the implantation of the titanium cylinders in the linea temporalis To diminish the risk of infection from the surgical procedures all the patients were given Lincomycin (Lincocin®), 0 5 g twice daily for 5 days No signs of local infection were found after any of the surgical procedures When the patients were checked one month after surgery they all had an intact tympanic membrane and hearing tests showed that in all the air/bone gap was closed within 10 dB

The tissue sections stained for histology on the bone from the tibia mould showed in 4 of the patients osteocyte lacunae containing vital looking cells The enzyme histochemical in



Fig 8 Not preformed bone taken from os temporale (*a*) The arrows on the top right side of the picture indicate an area where the bone h been sculpted by the burr While th osteocytes to the left (on the far sid of the surgical trauma) seem v tal the lacunae in the right part (unde the sculpted area) are empty throughout the preparation (*b*) Weak or no $NADH_2$ enzyme activ is found in the bone while the sof tissue away from the trauma. shows $NADH_2$ activity

vestigations showed activity of diaphorases and ATPase indicating viability of the bone and endothelial cells (Fig 6) Strong alkaline phosphatase activity and weak or no acid phosphatase activity were found in the grafts In the central part, separated by cement lines, there was a minor bone area with lacunae containing pyknotic nuclei This central part showed positive lipid staining indicating degenerative processes This bone also lacked diaphorase and ATPase activities

In the fifth patient, H F all the bone from the tibial mould taken for microscopic investigation showed empty lacunae lacunae with pyknotic cells and no diaphorase enzyme activities

The histological and histochemical invest gations of the bone from the os temporale c linder showed the same general pattern found in the tibia mould bone of the 4 patien described above The preformed bone fro tibia and os temporale was surrounded by enveloping sheath of connective tissue w strong enzyme activities This connective t sue sheath was somewhat thicker around t os temporale bone (Fig 7)

The cortical bone taken from the masto process and sculptured peroperatively had zone about half the width of the graft whe the osteocyte lacunae were empty or co tained pyknotic nuclei The entire gr showed weak or no enzyme activity (Fig 8)

DISCUSSION

he results obtained in clinical pilot studies I I II show that the findings in the animal iments are also valid for man It was thus ble to obtain a preformed autologous icle suitable for ossiculoplasty from a um mould placed in the proximal tibial ,is

In study I two of the ten chambers contained of suitable quality for ossiculoplasty In , II this proportion was improved to seven of ten It seems reasonable to assume that changes made in the latter study were re- ble for the better result but the limited of patients does not permit a detailed aluation of the importance of each single actor

A histological comparison between the ' bone from the tibial mould and the mastoid and a conventional, non ossicle graft from the temporal was performed The preformed bone ved higher density of osteocytes A higher *of the osteocyte lacunae of the pre-* ' bone contained nuclei than in the ossi- e grafts sculptured peroperatively In the from the chamber filled with spongy and marrow hematoma a marrow space surrounded by a cortical shell could be rec- gnized The same type of marrow cavity ould not be identified in the prosthesis where a piece of cortical bone had been placed at nsertion This ought to lead to a somewhat slower revascularization and also delay the other phases of integration of the graft

The histochemical investigation of dia- norase, ATPase and alkaline phosphatase en- zyme activities showed a strong activity in the preformed grafts This was valid for all pre formed grafts with the exception of the tibia mould bone from one of the patients that showed no enzyme activity at all

The fact that there was strong alkaline phosphatase activity and *weak or no acid* phosphatase activity in the preformed trans- plants indicated that bone formation domi nated over bone resorption (Magnusson et al , 1977)

The non viable small bone areas separated by cement lines found in the centre of the tibia *mould preparations were probably remnants* of the bone implants placed in the mould when it was installed in the tibia

The histochemical findings indicate that the cells of the preformed grafts were viable (" ac- tive") at the time of transplantation This is probably due to the fact that the surgical trauma (handling, vibration, heat) inevitable during a surgical procedure is much milder for a preformed graft than for a graft taken and prepared during the reconstruction of the os- sicular chain Thus, the properties which ought to make the preformed ossicle superior to that peroperatively sculptured are it is vital and it is covered by a fibrous lining

The procedure for producing a preformed ossicle in a titanium mould placed in the tibia is, however, somewhat too complicated to be used as a routine method In spite of the fact that no osteomyelitis was seen, this risk can- not be neglected and the special antiseptic ar- rangements put special demands on the sur- gical team The discomfort from the leg that most patients experienced should also be kept in mind

If a method is to be used in routine work it has to be simple and safe The procedure in study IB is a step in the right direction and could easily be integrated in a staged proce dure The anatomical region is also well known to every otosurgeon Although the number of patients used in this study was restricted it seems possible to get a high success rate with titanium cylinders in linea temporalis and the surgical technique could possibly be further refined The next phase of this investigation will be to take advantage of the experience achieved in the pilot studies Further pilot studies are, however, necessary and planned before this new concept in ossiculoplasty can *become a clinical routine procedure It is also* important to stress that the ultimate this new type of prosthesis can only be

after a long period of careful follow-up in a large number of patients

ZUSAMMENFASSUNG

Seit mehr als zehn Jahren ist ein Forschungsprojekt betreffend praformierter autologer Gehöhrknöchelchen im Gange. Nach Tierexperimenten folgten klinische Versuche Diese zeigten daß Knochengewebe in einer Titaniumform, die in die proximale Tibiametaphyse eingepflanzt wurde, produziert werden konnten In der vorliegenden Arbeit wurden noch fünf Patienten operiert Das experimentelle Muster wurde diesmal im Vergleich zu den fruheren klinischen Studien geandert, und eine größere Menge von Knochengewebe wurde erzeugt Außer den Tibiaformen wurden zehn Titaniumzylinder um eine Knochenbrücke in Linea temporalis eingebaut Neun von den zehn Zylindern enthielten für Ossickuloplastik geeignetes *Knochengewebe* Die *nachfolgende* histologische Untersuchung zeigte lebendiges Knochengewebe mit ausgefüllten Osteozytenlakunen Zur Zeit der Transplantation zeigten sich die Transplantatzellen bei der histochemischen Untersuchung lebendig Die Implantation der Titaniumzylinder im Schlafenbein war einfach, und belastigte die Patienten wenig Die Infektionsgefahr in diesem Gebiet konnte fast vernachlässigt werden

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AUDITORY FUNCTION AFTER HAEMOPHILUS INFLUENZAE MENINGITIS

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Abstract Eighty three children having recovered from *Haemophilus influenzae* meningitis were examined with audiometrical tests Fifteen of the children (18 1%) had significant hearing loss Bilateral severe hearing loss was found in 3 patients Three patients had severe hearing loss affecting one ear and slight or moderate hearing loss affecting the contralateral ear Six children had entirely unilateral severe hearing loss Bilateral or unilateral slight or moderate hearing loss was found in 3 patients The remaining 68 patients had normal pure tone average Half of these patients however showed minimal hearing impairment at the low and high frequencies

Haemophilus influenzae (HI) is one of the most common etiological agents of bacterial infections in childhood One of the most serious diseases caused by this species is acute purulent meningitis The disease is very uncommon in infants under 2–3 months of age, but over that age it is more frequently seen up to 3–4 years of age It is of importance to establish an early etiological diagnosis so as to minimize both mortality and post infectious sequelae

There is a high rate of long term sequelae after HI meningitis, even after adequate antibiotic treatment (Sell et al , 1972) Hearing loss seems to be one of the most common ...als This article presents the audiologi...l findings of a follow up study of patients with HI meningitis In a separate article (Lind... et al , 1977) the relationship between ...apy and sequelae will be discussed

MATERIAL

During the years 1968–75 altogether 97 individuals were treated for HI meningitis in the Goteborg–Molndal region in Sweden

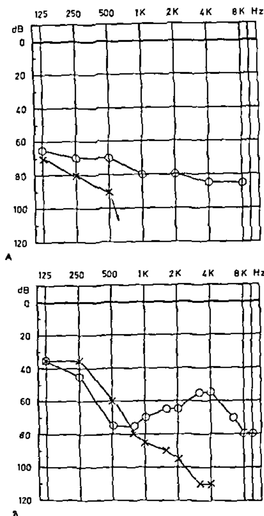
The patients were treated either at the Dept of Pediatrics or the Dept of Infectious Diseases, University of Göteborg, or at the Dept of Pediatrics County Hospital, Molndal

The etiological diagnosis was based on the demonstration of HI in cerebrospinal fluid and/or blood of all but 3 patients These latter 3 patients, however, had a fourfold or higher antibody titre increase against HI, as measured by a complement fixation test Three patients died intercurrently Eighty-three of the remaining 94 patients could be traced and examined with auditory tests These patients were tested from one month to seven years after the meningitis The age of the tested individuals at the time of the meningitis varied between 2 months and 22 years, only 3 patients being older than 15 years of age The mean age of the patients was 3 3 years and the median age 2 4 years

AUDIOLOGICAL METHODS

The audiological methods were selected according to the age and development of the

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ig 1 (A B) Pure tone audiograms of 2 boys three (A) d six (B) years of age with bilateral severe hearing oss

patients tested Sixteen children, between 6 months and 3–4 years old, were tested with visual reinforcement audiometry (Liden & Kankkunen, 1969) Twenty six children over 4 years of age were tested with play audio metry according to Barr (1955) Ordinary pure tone audiometry was used in 41 cases

Patients with a pure tone average (pta) better than 20 dB HL at 500–2 000 Hz in both ears were considered to have normal hearing Thirty three of the most cooperative patients with normal pta and older than 4 years were picked out in order to detect diminutive hear ing impairment All these patients had normal tympanic membranes The pure tone thresh olds from 125 to 8 000 or 10 000 Hz were measured Speech audiograms were performed in most instances

A control group consisting of 65 individuals was studied in the same way None of the individuals in the control group had any history of CNS infections or hearing disorder and there was no sign of middle ear disease at the time of the testing The age range of the individuals in the control group was 4 to 25 years (mean age 5 6 years, median age 4 7 years of age)

RESULTS

Fifteen of the 83 patients, 18 1% exhibited different degrees of sensorineural hearing loss (pta ≥20 dB HL)

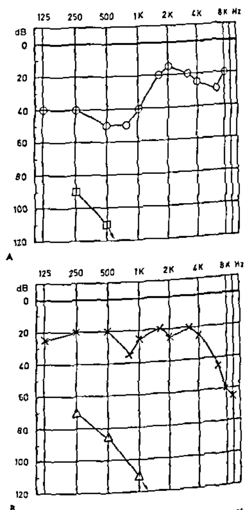
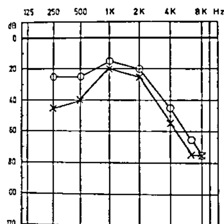


Fig 2 (A B) Pure tone audiograms of 2 patients six (A) and five (B) years of age with severe hearing loss in one ear and slight hearing loss in the *other ear*



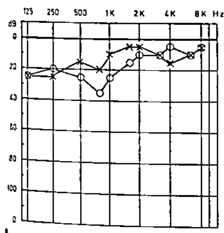


Fig 3 (A B) Pure tone audiograms of 2 patients two (A) and ten (B) years of age with slight hearing loss

Bilateral severe hearing loss

No patient was totally deaf but 3 had severe hearing impairment (pta >60 dB HL) affecting both ears. One of these patients was totally deaf in one ear and his other ear exhibited a hearing loss of 77 dB pta (Fig 1 A). The second patient had a hearing loss of 82 dB in one ear and about 70 dB in the other ear (Fig 1B). The third patient gradually developed a severe hearing loss starting about 6 months after the meningitis. His hearing deteriorated to 80 dB in one ear and to 62 dB in the other.

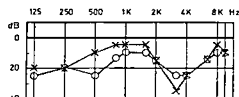
Unilateral severe hearing loss

Three patients had unilateral deafness or severe hearing loss combined with a slight or moderate hearing loss not exceeding 45 dB in the other ear. One of these patients had one deaf ear. His other ear showed a hearing loss of 40–50 dB up to 1 000 Hz and of 15–35 dB from 1500 Hz to 8000 Hz (Fig 2 A). The second patient had also one deaf ear and a slight hearing loss of 20–35 dB up to 4 000 Hz and a high-frequency loss of 60 dB at 8 000 Hz in the other ear (Fig 2 B). The third patient showed a pure tone average of 65 dB in one ear and of 30 dB in the other ear.

The largest group, consisting of 6 patients, had unilateral severe hearing loss with normal hearing in the other ear. Five of these children had unilateral deafness and one patient had a unilateral hearing loss of about 80 dB.

Slight to moderate hearing loss

One patient exhibited a slight sensorineural hearing loss, affecting both ears, with a pta of 20 and 28 dB, respectively. Both ears also showed a high frequency loss of 65–75 dB at 6 000–8 000 Hz (Fig 3 A). Two other patients had a slight unilateral hearing loss. One of them had a pta of 22 dB with a dip of 35 dB at 750 Hz (Fig 3 B). Tympanometry and stapedius reflex test were normal. The other had a unilateral hearing loss of 23 dB at 500–2 000 Hz and a high frequency loss with a maximum of 60 dB at 8 000 Hz.



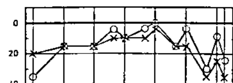


Fig 4 (A B) Minimal hearing impairment after HI meningitis found in 2 patients ten (A) and nine (B) years of age

Occurrence of otitic meningitis

An otological examination was performed in the course of the meningitis in 10 of the 15 patients with hearing loss The tympanic membrane was normal in 7 cases while a slight redness was noticed in 2 cases One patient was admitted to the hospital with purulent acute otitis media and with signs of purulent meningitis This patient was the only one in whom the meningitis might be considered secondary to acute purulent otitis media He became completely deaf in the otitic ear (Fig 2 A)

Minimal hearing impairment

Sixty eight patients had pta better than 20 dB Of these individuals, 33 were picked out in order to reveal diminished hearing Sixteen of these latter patients had pure tone thresholds better than 20 dB at all frequencies tested (125–8000 or 10000 Hz) The remaining 17 individuals exhibited normal hearing capacity at the speech frequencies (500–2000 Hz) They had, however, slight deficiencies reaching or exceeding 20 dB at two or more frequencies outside the speech region

In many of these cases with minimal hearing deficiencies, the pattern of the pure tone threshold had a serrated appearance In the low and high frequencies a few, often sharp dips were found, surrounding a plateau of normal hearing at the speech frequencies (Fig 4 A, B)

DISCUSSION

A profound bilateral sensorineural hearing loss is a severe residual after HI meningitis In our study 3 of the 83 children (3 6%) showed such hearing loss This figure is comparable to the incidence of bilateral severe hearing loss reported by other authors Sell et al (1972) found a corresponding figure of 1 8% Gamstorp & Klockhoff (1974), in their study, reported an incidence of severe hearing loss of at least 3 5% In our materials, however less disabling hearing impairments were more common than bilateral pronounced hearing loss A considerable discrepancy in hearing between the two ears was found in many patients A profound unilateral hearing loss combined with only a slight or moderate impairment in the opposite ear was found in 3 cases (3 6%) Still more common was a unilateral profound hearing loss, with normal hearing capacity in the opposite ear, which was found in 6 patients (7 2%) The occurrence of unilateral hearing loss after meningitis has been reported earlier (Grove & Fox, 1949, Trolle 1950, Teng et al , 1962, Sell et al 1972 Landthaler & Andrieu Guitrancourt 1973 Johannsen, 1974, Svenungsson et al 1976) Sell et al (1972) found unilateral hearing loss in 8 9% of their cases and Svenungsson et al (1976) in 9% in their study These results are thus well in accord with the incidence found in the present investigation Including 3 cases of slight to moderate hearing loss (one bilateral and two unilateral) the overall incidence of sensorineural hearing loss after HI meningitis was 18 1% in this material Sell et al (1972) reported a higher incidence of hearing loss, 33 9% The incidence of severe hearing loss, both bilateral and unilateral was 14 4% in the present study This figure does not differ markedly from the corresponding incidence reported by Sell et al (1972) Thus in their study, a majority of the patients with hearing loss had only non significant auditory impairment Many of their cases were probably similar to those in our study with normal pta in connection with slight hearing impairment in the low and high frequencies This auditory pattern fits at least 2 cases of non-significant hearing loss in the study of Sell et al The inclusion of such and similar patients might account for the higher incidence of hearing impairment reported in their study In the present study as many as half of the patients (51 5%) with normal pta exhibited such minor defects In the control group similar defects were found in only 10 8%

Evidently, even those who escape signi cant hearing loss after HI meningitis often how minor hearing impairment These de- however, are so trifling that they do ot constitute a social handicap, and they may iot even be noticed by the individuals af

Since hearing disorders are commonly l after HI meningitis, all children who ave suffered from this disease should be ex ined with hearing tests A pure tone audio- should be performed shortly after re- overy However, hearing impairment may long after the meningitis, and so the t should be repeated about 6 months after disease

ZUSAMMENFASSUNG

Dreiundachtzig Kinder die nach *Haemophilus influenzae* Meningitis genesen waren wurden mit audiometrischen Testen untersucht Funfzehn der Kinder (18 1%) hatten einen merkbaren Gehörschaden Doppelseitige schwere Gehorschaden wurden bei drei Patienten gefunden Außerdem hatten drei Patienten einen schweren Gehor schaden an einem Ohr und einen leichten Gehorschaden am anderen Sechs Kinder hatten nur einseitige grave Schwerhörigkeit Ein leichter Gehorschade doppelseitig oder einse tig wurde in drei Fällen gefunden Die ubrigen 68 Patienten hatten normale Audiogramme Die Halfte d eser Pat enten hatte aber einen minimalen Gehörscha den was die niedrigen und hohen Frequenzen anbelangte

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EPITHELIAL MIGRATION ON THE TYMPANIC MEMBRANE

An Experimental Study

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Abstract The existence of an epidermal migration pattern was studied in mice using a whole mount autoradiographical technique The mitotic activity appeared to be confined to an area close to the annulus tympanicus A very slow migratory activity of the labelled cells was observed situated in the basal cell layer Perforation of the tympanic membrane showed no effect on the position of the labelled cells This slow migration was considered to differ from the rapid epithelial migration described by several authors The latter migration was suggested to represent a mechanism for cleaning the tympanic membrane

The existence of an epidermal migration pattern on the human eardrum was suggested as early as 1877 by Burnett and confirmed by Buck (1880) and Bezold (1908) They supposed that this cell migration might explain the self cleaning property of the external meatus This phenomenon was described in greater detail on the human tympanic membrane with the use of ink dots by Stinson (1936)

From comparable experiments, Magnoni (1938) concluded that cholesteatoma of the middle ear could not originate from the inward migration of epithelium from the external meatus, since no migration of epidermal cells was noted in this direction In contradiction Simmons (1964) advocated the use of the ink dot marking technique as a clinical test for the detection of cholesteatoma formation in central perforations A careful analysis of ink dot migration patterns and rates in human tympanic membranes was made by Alberti (1964) He noted an average migration rate of 0 0[illegible] mm per day, while the umbo appeared to be the centre of migration The latter author also doubted on the existence of any relationship between epidermal migration and the origin of middle ear cholesteatoma because such migration would tend to prevent rather than cause such an epidermal cyst Tomasetti (1965) Franz (1966) and Gulzow (1973) arrived at same conclusions with comparable experiments

Using a radioactive tracer and DNA-precursor (H^3 thymidine) Litton (1968) identified and traced the route of the labelled nuclei of the squamous epithelium of the eardrum and external auditory canal of guinea pigs He concluded from his experiments that in guinea pigs the epidermal migration occurs by a differential growth rate in an area of the auditory canal wall just distal to the annulus tympanicus This very rapidly proliferating generation centre should maintain a thin pliable lining over the vibrating tympanic membrane

Similar experiments on traumatic eardrum perforations (Clawson & Litton 1971) give support to this hypothesis of a differential growth rate from a generation centre

Apart from these observations several questions concerning this migration phenomenon remain and require further research The aim

this paper therefore is to discuss results of nes of experiments on this migration phe omenon with the use of autoradiography

METHODS

k dot marking

nall indian ink dots were placed at various cations on the tympanic membrane and the in of the external auditory canal of adult amsters (20 animals)

For this marking procedure, using the perating microscope, the animals were an sthetised with nembutal (60 mg/kg, admin tered intraperitoneally)

utoradiography

he isotope experiments were performed on our series, each consisting of 8 Swiss albino ice With the use of the operating micro ope a small perforation was made in the ght eardrum of the anaesthetised animals he left ear served as control Immediately fter perforation, the animals received 1 μCi H^3 thymidine/g body weight administered in peritoneally

In order to exclude any possible influence of he diurnal rhythm on the final results one roup animals was injected at 12 00 hours and nother group at 24 00 hours The animals ere sacrificed at different intervals varying rom 2 hours up to 18 days The tympanic membranes were dissected, fixed in neutral ormaldehyde and placed on gelatinized slides, with the epidermal layer upside

After dehydratation and cleaning in xylol lcohol solutions the slides were coated with a uclear emulsion (NTB_2, Eastman Kodak) sing the dipping technique (Kopriwa & Le lond 1962) The specimens were stored in ightproof boxes for 2–4 weeks and subse quently developed, fixed and stained with hematoxylin–eosin

The exact localization of the labelled nuclei registered using a lateral drawing tubus, ixed on the microscope Only those cells here the deposit of silver granules was definitely separable from the background were scored

Apart from the use of H^3 thymidine, similar experiments were performed with the use of 5-iodo-2 -(deoxy)-6H^3 uridine (IDU) Thymidine is in fact not an ideal DNA precursor in long term turnover experiments, because of the re utilization and re incorporation of labelled breakdown products This is not the case for IDU, where the methyl group in position 5 is replaced by iodine Once incorporated, IDU remains fixed in the DNA molecule through the whole lifetime of the cell, and re utiliza tion after cell-death is minimal and ineffective According to Commerford (1965) and Feinen degen et al (1966) IDU is an excellent substance for turnover experiments in the long term

One disavantage is the fact that IDU is less easily incorporated than thymidine (Hughes et al , 1964)

In two series, each consisting of 6 mice, the animals received 5 μCi IDU per gram body weight, injected intraperitoneally The animals were sacrificed at different intervals and their tympanic membranes were studied according the technique used for H_3 thymidine

RESULTS

Ink dot marking

The results of this preliminary experiment were disappointing Because of the rapid disappearance of the dots, no migratory pattern could be observed In 76 ears studied with this method the ink dots had already disappeared after one or two days

Autoradiographs

The results obtained from normal tympanic membranes are summarized in the drawings of the tympanic membranes (Figs 1 and 2) Each dot in these figures represents one labelled cell The highest mitotic activity in the pars tensa is mainly situated near the annulus tympanicus and the manubrium mallei

In the pars flaccida no specific localization

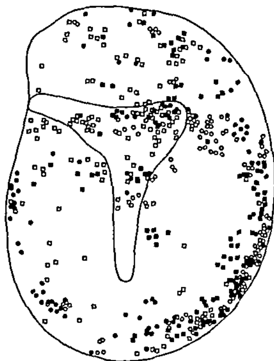


Fig 1 After 2 hours (●) after 1 day (○) after 2 days (□) after 4 days (■)

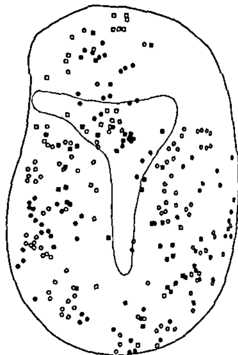


Fig 2 After 8 days (○) after 12 days (●) after 14 days (□) after 18 days (■)

of the labelled cells was found During an observation period of 18 days no clear migration pattern could be found Only a slight tendency of the labelled cells to move slowly from the periphery to the centre of the pars tensa was observed With progression of time the labelling became more difficult to identify because of the dilution of the isotope by cell division or physiological destruction

The data obtained with the perforated tympanic membranes (Fig 3) clearly show that no essential difference can be observed in the isotope distribution in comparison with the control ears The perforations seem to close without any tendency of the labelled cells to migrate towards the perforation

The results obtained with H^3-IDU appeared to be fully comparable to those after H^3-thymidine administration (Fig 4)

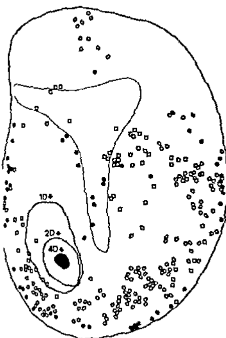
DISCUSSION

According to various authors there undoubtedly exists a migratory activity of epidermal cells on the human tympanic membrane (Stinson, 1936, Magnoni, 1938, Simmons, 1961, Litton, 1963, Alberti 1964, Tomasetti 1965 Frank, 1966, Gülzow, 1973)

Several mechanisms underlying this migration have been postulated, such as the vibration of the tympanic membrane or the blood flow in the vessels of the membrane (Link 1952)

According to Litton (1968) and Clawson & Litton (1971) a generation centre of epithelial cells, localized in the external meatus and close to the annulus, should be the driving force for this migration phenomenon in guinea pigs It takes about 13 days to cross the tympanic membrane completely According to the same authors a comparable mechanism should exist in human

In contrast to the findings of these authors our results failed to show the existence of such a rapid epithelial cell migration in mice Only a very slow movement of labelled cells was observed from the annulus towards the central parts of the pars tensa Even after 18 days



g 3 After 1 day (●) after 2 days (O) after 4 days)

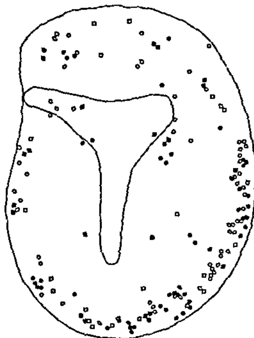


Fig 4 After 2 hours (O) after 2 days (●) after 4 days (□) after 8 days (■)

any labelled cells are still distributed arbitrarily on the whole tympanic membrane

These experiments reveal the existence of a entre of high mitotic activity in the annular rea and confirm the findings made by Reyen & Kuijpers (1971) The slow migration ate appeared to be mainly confined to the ells of the basal epithelial layer It seems unkely however, that this migration is com arable to the migratory activity described by itton (1968) and Clawson & Litton (1971)

It is highly likely that the observations made y Litton in guinea pigs (1968) represent the migration of a very superficial layer of the pithelium One argument in favour of this ypothesis is the similarity of the results they btained in using Indian ink labelling and H^3 hymidine With ink labelling only the upper art of the stratum corneum can be marked Furthermore the thickness of the tympanic membrane of the guinea pig varies between 20 and 40 μm and can increase to more than 100 after perforating Because the penetration H^3 radiation is maximally about 8 μm, labelled cells in the basal cell layer of the guinea pig tympanic membrane cannot activate the emulsion layer in whole mount autoradiography Therefore the observed blackening in the emulsion must originate from the super ficial layers of the epithelium In mice, however, the thickness of the membrane varies between 5 and 10 μm So the radiation from labelled basal cells in our experiments will ac tivate the emulsion in whole mount autoradiography The results obtained clearly demonstrate that perforation does not influence the position of the labelled cells to any great ex tent

The migration of the upper layer of the stra tum corneum might represent a physiological mechanism taking care of the removal of kera tin and cerumen from the tympanic membrane The displacement and even extrusion of eargrommets might be at least partly attributable to this mechanism The driving force of this cleaning process remains a matter of speculation, however

(#6757)

DPR+OKN

DPR+OKNRD

Fig 1 This figure shows nystagmus recordings of one squirrel monkey in two different conditions rotation in light (DPR+OKN) and rotation with a mirror in light (DPR+OKNRD) In this monkey the turning points of nystagmus direction (*arrows*) are clearly delayed (phase lag) in DPR+OKNRD but nystagmus rhythm is almost regular

tibular stimuli in order to investigate possible mechanisms associated with this phenomenon

METHODS

Healthy adult male or female squirrel monkeys (body weight 450–700 g) were used Rotatory stimuli were provided with a pendular rotation table suspended by a stainless steel wire from a 4 meter high ceiling When the table was rotated 180° and released, one oscillation took 15 5 sec Eighteen cycles were obtained in this manner with a gradual damping motion observed from the initial release The maximum angular acceleration of the initial oscillation produced by the present apparatus was 29°/sec², and the maximum angular speed was 72°/sec

To provide pendular rotation and optokinetic stimuli simultaneously, a 1 meter diameter white cylinder with 16 black stripes (3 5 cm wide) was installed around the pendular rotation table To eliminate peripheral vision during optokinetic stimulation, a head cover with an opening in front of the subject's eyes was used

Visual–vestibular conflict was accomplished by reversing the direction of the optokinetic stimulus with a mirror installed at a 45° angle in front of the monkey s eyes Because the distance between the eyes and the cylinder surface became longer and also the angle between the subject s visual axis and the cylinder surface became obtuse, the optokinetic stimulus magnitude was reduced to approximately 70%

Horizontal and vertical eye movements were recorded on a Beckman dynograph through a d c amplifier The position of the pendular rotation table was recorded by a potentiometer which was installed at the bottom of the table

Each monkey was secured in a laboratory built restrainer, 0 5 mg/kg amphetamine was injected intramuscularly, and the test was begun about 15 min later

Testing was comprised of three conditions First, vision was excluded and damped pendular rotation (DPR) was applied Second the monkeys were exposed to both rotation and the optokinetic stimulus rotation in light (DPR+OKN) Third, each subject experienced pendular rotation with the visual field reversed, rotation in light with a mirror (DPR+OKNRD) The tests were performed following the above described order of 1 through 3, one test a day each, except for the training phase in which DPR+OKNRD was repeated twice a day

The first part of the study covers the analysis of DPR+OKNRD and comparison between DPR+OKNRD and DPR Furthermore the training effect and effect from vestibular deafferentation (labyrinthectomy or lateral

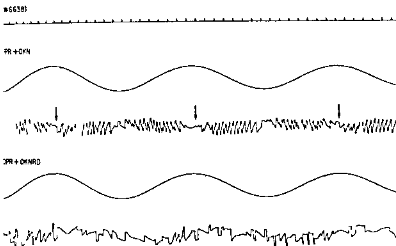


Fig 2 This subject shows very irregular saccadic or slow eye movement in rotation with a mirror in light (DPR+OKNRD) It is impossible to decide direction changing point of nystagmus

semicircular canal plugging) in DPR+OKNRD situation were studied

Upon the completion of the functional experiments, all of those operated animals were perfused intracardiacly and serial sections of the temporal bones were prepared according to standard procedure

RESULTS

DPR+OKNRD

The purpose of this part of the experiment was to evaluate the squirrel monkey's response to vestibular–visual directional conflict situation In order to eliminate any training or habituating effect, the data analysed for this portion of the study were taken from the initial trials of 17 squirrel monkeys only

In those monkeys who could resolve the vestibular–visual conflict, the direction of nystagmus response was determined by the optokinetic stimulus The table's turning point and nystagmic direction changing point were fairly well matched, and nystagmic rhythm was regular

On the other hand, those monkeys who could not pursue the visual target properly showed a phase lag between the table's turning point and nystagmic direction changing point (Fig 1) In addition, many of these subjects exhibited irregularity in nystagmic rhythms (Fig 2) Many also floundered, indicating that the condition was stressful to them

Among the total of 17 subjects in this experimental group, 2 demonstrated very little phase lag, or irregularity of nystagmic rhythm Eight exhibited either relatively large phase lag but with good nystagmic rhythm *or* minimal phase lag with considerable irregularity in the nystagmic rhythm The remaining 7 showed either very large phase lag, *or* very severe irregularity in nystagmic rhythm Therefore, 15 out of 17 (88%) showed poor target pursuing ability, when the directions of optokinetic stimulus and vestibular stimulus were conflicting

The phase lag and the irregularity of nystagmic rhythm did not necessarily show an identical tendency The range in phase lag among different monkeys is shown in Fig 3 As a trend, when the stimulus magnitude was reduced through the damped oscillation of the table, the phase lag became less However, the degree of phase lag reduction was not necessarily parallel to that of stimulus magnitude reduction

Those monkeys with good target pursuing ability (in DPR+OKNRD) showed minimal phase lag even at the first oscillation In contrast, subjects with poor target pursuing ability showed considerable phase lag even when

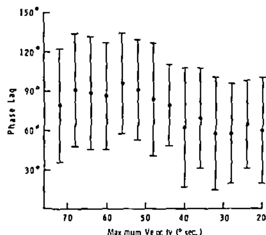


Fig 3 The means and standard deviations (17 squirrel monkeys) of phase lag in DPR+OKNRD *Abscissa* phase difference between direction changing points of table and nystagmus *Ordinate* maximum stimulus velocity of each oscillation

stimulus magnitude became small In many of those, bi directional irregular eye movements or nystagmic wave forms were observed

According to this result, the monkeys who could pursue the visual target in DPR+OKNRD condition were rather exceptional when compared with the majority which could not overcome the vestibular–visual directional conflict, even though the vestibular input was minimal On the other hand, during ordinary *pendular rotation in light (DPR+OKN), all* monkeys showed no difficulty in pursuing the visual target with no phase lag or irregular nystagmic rhythm All monkeys in this series showed good vestibular nystagmus in DPR (without vision), therefore all of them had good vestibular end organ functions

Comparison Between DPR+OKNRD and DPR

It is now known that the crista ampullaris lateralis acts as a change of speed receptor in response to sinusoidal rotation within a certain periodicity (Melvill Jones & Milsum, 1971) The vestibular–visual conflict situation in this experiment consisted of not only opposite directions of visual and vestibular stimuli, but also included the ratio between the two stimulus magnitudes Among the 1 squirrel monkeys, 8 were randomly selected for studying the relation of strength of vestibular nystagmus and pursuing ability of OKNRD The average eye speed from these 8 monkeys when rotated in light (DPR+OKN) or in dark changed linearly (Fig 4) and the variances in both conditions were somewhat similar (Open circles in Fig 4 represent the data taken from a subject with complete lateral semicircular canal block on one side and incomplete canal block on the other side) The average eye speed of DPR in each oscillation was between 53 3 and 73 8% (average 65 1%) of that of DPR+OKN This value reduced according to the stimulus decline. *When a mirror* was installed (DPR+OKNRD) the optokinetic stimulus speed reduced to about 70% of the stimulus speed without a mirror Thus, the eye speed of DPR should be about 93% (65 1%×100/70) of that of DPR +OKNRD, if the monkeys were able to pursue the OKNRD well

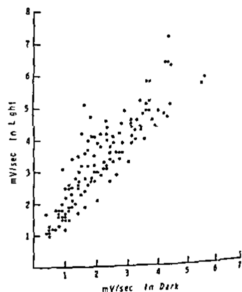


Fig 4 This scatter graph shows slow phase eye speed for comparing the strength of vestibular nystagmus (DPR (DPR *abscissa*) and optokinetic nystagmus (DPR+OKN *ordinate*) Closed circles represent 8 normal animals Open circles are recorded from the monkey in which one lateral semicircular canal is completely blocked whereas the contralateral one is incompletely blocked.

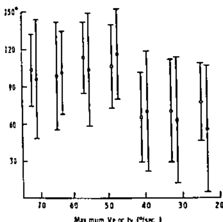


Fig 5 The means and standard deviations of phase lag obtained from 6 monkeys for the study of training effects Open and closed circles represent the first and last trials respectively

When the nystagmic eye speed in DPR, and that in DPR+OKN situation were compared in each individual monkey, the former was 49 3–89 5% of the latter The interindividual variance was large Therefore, when the vestibular provoked nystagmic response and the target pursuing ability in DPR+OKNRD situation were compared no correlation existed between those two

Training Effect

For this part of the experiment 6 squirrel monkeys with poor visual target pursuing ability in DPR+OKNRD situation were selected and the test trials were performed twice a week (2 tests per day) for 5 weeks (total 20 tests) in order to investigate if any functional improvement existed As was stated in the previous section amphetamine was administered to maintain monkeys alert during this sort of unnatural stimulus condition. To avoid the amphetamine s cumulative effect training trials had to be spaced no more than twice a week

One subject in this group showed slight improvement (17%) one subject s response became worse while the others exhibited basically no difference between the first test and the last test Inter individual variance increased (Fig 5) The monkey which showed some improvement also showed variable results and did not depict any gradual shortening of the phase lag

Vestibular Deafferentation

A Labyrinthectomy

Three squirrel monkeys (randomly assigned) received bilateral labyrinthectomies (two staged) Four months after unilateral labyrinthectomy (first stage) a very weak spontaneous nystagmus was still observed when the subject was placed in the dark (with amphetamine injection) and slight directional difference (and a reduction of phase lag) was found in the DPR+OKNRD test situation (Fig 6) When the direction of spontaneous nystagmus and OKNRD matched the pursuing ability was slightly improved

Immediately after the second stage labyrinthectomy (about four months after the unilateral labyrinthectomy) the phase lag previously exhibited in the DPR+OKNRD test situation was clearly reduced or even disappeared (Fig 6) However a reduction of nystagmic eye speed was also noted in this situation

The eye speed of the vestibular nystagmus

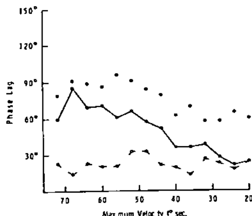


Fig 6 This figure shows the decrease of phase lag (meaning better optokinetic pursuit) in DPR+OKNRD in labyrinthectomized subjects Closed circles are the mean of 17 intact monkeys Open circles connected by solid and interrupted lines represent the post unilateral and bilateral labyrinthectomy status respectively

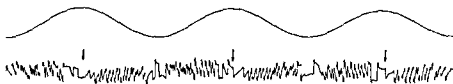




Fig 7 Nystagmus recordings of the monkey in which both lateral semicircular canals are completely blocked Optokinetic pursuit in DPR+OKNRD perfect and phase lag appeared

(DPR) *after the unilateral labyrinthectomy* reduced to about 60%, and after bilateral operations the DPR nystagmus was abolished In the test situation of DPR+OKN, the eye speed was not changed after the unilateral labyrinthectomy, but was lower after the bilateral labyrinthectomies, as the pursuing ability declined due to the disappearance of the vestibular inputs

B Lateral semicircular canal blocking

Another 3 squirrel monkey subjects (randomly selected) were used for this part of the experiment The tests were performed 1 week and 2 weeks after unilateral lateral semicircular canal plugging Four weeks after the first operation the contralateral canal blocking was performed Thereafter, the test was similarly performed 1 week and 2 weeks after the second operation

The eye speed of vestibular nystagmus evoked by DPR after unilateral operation was about 60% of that observed pre operation (somewhat similar to the situation after the unilateral labyrinthectomy) Following the blocking of the contralateral lateral semicircular canal the DPR evoked nystagmus completely disappeared, except for 1 monkey which had incomplete obliteration of the semicircular canal on the second operation side Histological examination indicated that in 2 animals bilateral horizontal semicircular canal plugging was complete, while in the third animal, the contralateral (second operation) canal block was incomplete Nystagmus provoked in the test situation of DPR+OKN showed no obvious difference between pre operation, post unilateral operation and post bilateral operation (3 monkeys)

The 2 squirrel monkeys with the

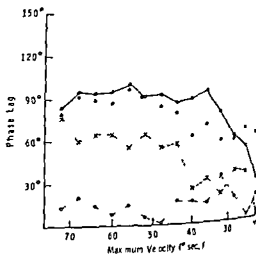


Fig 8 This figure shows the phase lag decrease in bilateral horizontal semicircular canal blocking in OKNRD test Open circles connected with soli... rupted lines represent the post unilateral and bilat... ...pectively One monkey ...mpletely blocked ...al is not...

lateral lateral semicircular canal plugging, owed significant improvement of target ng ability and a disappearance of phase g (in the test situation of DPR+OKNRD) ig 7) This change was not observed following the unilateral operation (O—O in Fig) In the incomplete obliteration monkey, he eye speed of vestibular nystagmus (DPR) as about 38% of that of the optokinetic ystagmus (DPR+OKN) (O in Fig 4) This icular value was lower than the lowest alue (49 3%) observed in the 8 normal control subjects However, the target pursuing bility was still incomplete in the test situation of DPR+OKNRD, and the result (×---× Fig 8) was found just in between unilateral canal blocking and bilateral canal blocking (O O)

Unlike the post bilateral labyrinthectomy ondition the results from the bilateral lateral emicircular canal block indicated that the postoperative eye speed in the DPR+OKN est situation did not decline The target pursuing ability in the DPR+OKNRD test was ound to be good after successful bilateral anal block (Fig 7)

DISCUSSION

Results from the present experiment indicate hat the majority of normal squirrel monkeys employed could not control unfavorable vestibular input when its direction was in conflict with that of visual information (DPR+OKNRD)

As far as the visual stimulus is concerned, he maximum angular acceleration produced by this DPR apparatus was 29°/sec² and the maximum angular speed was 72°/sec, which should not be difficult for squirrel monkeys to pursue visually As was found in the animals with bilateral semicircular canal blocking, no difficulty was encountered in pursuing the visual field without having dynamic semicircular canal inputs in the test situation of DPR+OKN

Fukuda and others (1957), using rabbits, reported vestibular–visual coordination by exhibiting the improvement of the visual target tracking ability with the supplementation of 1°/sec² angular acceleration to the vestibular apparatus Later, Veenhof (1965) found similar results by supplementing linear accelera tion However, it should be considered that the rabbit, having laterally positioned eyes, has a different oculomotor system compared with that of the primate

When optokinetic stimulus is presented with an angular acceleration of 1°/sec², the adaptation limit is somewhere around 120°/sec in the squirrel monkey Except for the fact that the stimulus was sinusoidal in nature, the maximum stimulus speed of 72°/sec in the present DPR stimulus is definitely below this limit, and therefore, as was observed monkeys should not have any difficulty in tracking the target On the other hand, only two subjects could pursue the visual target in the test situation of DPR+OKNRD, and inter-individual variance was remarkably large among all monkeys tested Even in poorly pursuing monkeys, vestibular nystagmus was modified in this test condition suggesting that visual input is dominant

Gonsher & Melvill Jones (1976) reported that when prisms were installed in front of the eyes, in order to reverse left–right directions in humans and cats, the subjects showed poor target pursuit ability at the early stage when sinusoidal rotation was employed At least in the initial stage of direction reversal, vestibular–visual conflict clearly existed in both humans and cats

Guedry (1968) studied the relationship between the visual acuity and vestibular stimulation or vestibular nystagmus, and reported the existence of dominant inter individual variance of visual acuity decline He also noticed the correlation between the degree of vestibular nystagmus in light and visual acuity during the rotation The degree of visual input dominance over the vestibular evoked nystagmus showed a large inter individual variance From our observation in squirrel monkeys,

no clear relationship existed between the target pursuing ability in DPR+OKNRD test and the degree of vestibular evoked nystagmus (DPR) It is considered that target pursuing ability is dependent on the degree of visual input dominance over the vestibular input

Young et al (1973) reported that the circular vection was enhanced when the directions of vestibular and visual stimuli matched, and contrarily circular vection was reduced when the directions did not match Under a direction matching situation, the responses were summated, however, when the stimuli conflicted, the qualitative nature of the response changed Although circular vection and nystagmus are not exactly similar responses, our experiment demonstrated one aspect of vestibular–visual conflict

Under the present experimental situation of DPR+OKNRD, the qualitative change of the nystagmus was quite common in addition to the phase lag Irregular change of nystagmic direction and irregular eye movement (wave form) were commonly observed The former may represent non linearity in the management of conflicting stimuli, while the latter may represent the central confusion failing to manage direction contradicting inputs

It is generally accepted that a visual stimulus is dominant over a vestibular stimulus It is known that ballet dancers and figure skaters can inhibit post-rotatory nystagmus by visual fixation, but this ability is acquired after training Untrained people usually cannot suppress vestibular reaction well In their chicken rotation experiments, Fukuda and others (1958), reported that in order to control vestibular inputs, transition occurred in posture from a passively rotated condition to an actively adjusting status after repeated rotatory training In such active postural adjustment, muscle and joint inputs should also make an active contribution

The test situation of DPR+OKNRD, visual stimulus and vestibular stimulus were given in conflicting directions, was an unnatural stimulus situation for the body However even under this sort of conflicting situation as Gonsher & Melvill Jones (1976) exhibited adaptation could still occur The inhibition of vestibular nystagmus and change in phase shift were observed This was based upon somewhat altered stimulus management mode which could be considered as a system plasticity The repeated application of optokinetic stimulus resulted in response enhancement; however, in the situation of vestibular–visual conflict, which was an unfavorable and unexperienced condition for the body, adaptation may not occur easily

As was seen in our one case with unilateral complete semicircular canal block and contralateral incomplete canal block, even though the vestibular input was much reduced the vestibular–visual conflict could not be controlled easily in the test situation of DPR+OKNRD This result indicates that the conflict could be established even by limited vestibular input

The different control ability of vestibular–visual conflict among different squirrel monkeys was observed in the present experiment Two monkeys with good pursuing ability in the present series showed good OKN tracking ability, good DPR nystagmus, and good post rotatory nystagmus One interesting finding in those was that the duration of post rotatory nystagmus and optokinetic afternystagmus was relatively long and the declining pattern was very gradual It is not known whether the ability to control vestibular–visual conflict and system adaptability are similar in nature or not, but the control of vestibular–visual conflict might be something different from the ability obtained through repeated training even though the mode of training could be another crucial factor

ZUSAMMENFASSUNG

Im Totenkopfaffen wurde der Richtungskonflikt zwischen den vestibularen und visuellen Reizen untersucht. Der Affe wurde sinusförmig gedreht und die Richtung seiner Zielverfolgung wurde vor seinen Augen umgekehrt [illegible] Einbaus eines Spiegels auf einer 45° Ebene Eine [illegible]

duelle Abweichung wurde beim Kontrollieren estibular-visuellen Konflikts bemerkt Der Grad des bular-visuellen Konflikts und der Reizgroße hatte lineare Beziehung Gleichfalls bestand keine Be-hung zwischen dem vestibular-visuellen Konflikt und Anreizung des vestibularen Nystagmus Wahrend es Zustandes des vestibular-visuellen Konflikts ver-esserte sich die visuelle Zielverfolgung nicht, wenn mal am Tage Versuche unternommen wurden, und war zweimal in der Woche, auf die Dauer von fünf vochen Die Verminderung der vestibularen Zufuhr, ie durch Operation geschaffen wurde, führte zu einer erung des vestibular hervorgerufenen Nystag mus und des vestibular-visuellen Konflikts Deren Grade waren jedoch nicht unbedingt parallel Ein interindivi Unterschied in der Reaktion auf den vestibular-visuellen Konflikt wurde auf die vestibular-visuelle Ko-ordination bezogen

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NEURAL DISCHARGE OF MEDIAL GENICULATE BODY UNITS AND SINGLE SEMICIRCULAR CANAL STIMULATION

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Abstract In curarized guinea pigs 68 neurons of the medical geniculate body (MGB) were tested with vestibular and acoustic stimulations Single semicircular canals were stimulated thermally Convergence of acoustic and vestibular afferences on the same MGB unit was observed Following stimulation of the semicircular canals activation and inhibition of unitary discharge were recorded inhibition being predominant while when clicks were delivered bursts of activity occurred The implications of MGB in vestibular and acoustic integration are postulated

Attempts have been made to define, for several species, specific thalamic cell groups carrying vestibular information to vestibular cortical fields identified within the somatosensory field S I and within a parietal field in carnivores and primates (Fredrickson et al , 1974, Liedgren & Schwarz, 1976, for recent reviews) Cells responding to vestibular stimuli appeared to be scattered over large thalamic areas, known for other functions (Copack et al , 1972, Deecke et al 1973 1974, Gernandt, 1950, Hassler, 1948 1964 1972, Mickle & Ades, 1954, Raymond et al 1974, Sans et al , 1970, Spiegel et al , 1965 Wepsic 1966), the ventro basal and posterior thalamic nuclei, however, seem to receive relatively direct vestibular projections (Deecke et al 1974, Liedgren & Schwarz, 1976 Liedgren et al , 1976)

In the guinea pig, the vestibular cortical field is located within the somatosensory fore limb area S I (Ödkvist et al , 1973) but the thalamic stations on the vestibulo-cortical pathway have not been clearly identified although vestibular projections have been described within the *nucleus parafascicularis* lateral and medial geniculate bodies (Gerebtzoff, 1950) The medial geniculate body (MGB) receives the greatest number of vestibular fibres and, consequently, it has been proposed as the major vestibular projection area

The purpose of this study was to verify the vestibular representation in MGB and to analyse the possible convergence of inputs from single semicircular canals on these diencephalic neurons Acoustic and vestibular convergence was also investigated Previous studies on other structures connected with the vestibular system (lateral vestibular nucleus, paramedian pontine reticular formation, oculomotor nucleus nystagmogenic cortical area) have been carried out in the guinea pig and various patterns of convergence of ampullar afferences have been demonstrated following thermal stimulation of single osseous canals (Desole & Pallestrini, 1969, Mannu & Desole, 1966, Pallestrini & Desole 19[illegible] Petrosini et al , 1975)

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ig 1 Electrolytic lesion of a recorded site in the left medial geniculate body (*MGB*) (*arrow*) *rn* red nucleus *III* oculomotor nucleus Magnification ×10

METHODS

Twenty guinea pigs of both sexes, weighing 350–500 g were used in the present research The animals were initially anaesthetized with ether, then tracheotomized and fixed in the prone position in a stereotaxic apparatus with the head tilted slightly down The animal were maintained throughout the experiment at 37–37 5°C temperature by heat lamp and pad The ECG was monitored by means of needle electrodes inserted into the left and the right forelimbs The heart rate was roughly 160 beats/min initially, with a tendency to fall slightly as the experiment progressed Two holes were drilled bilaterally in the temporal bones the first rostro dorsally and the second just posterior to the external auditory meatus, for epitympanic recessus and *bulla tympanica* opening Thus, it was possible to expose the ampullae of the lateral and anterior semicircular osseous canals in the epitympanic recessus and the prominence of the posterior semicircular osseous canal on the medial wall of the *bulla tympanica* Thermal stimulus was applied, employing the technique of Lorente de No (1931), on the single osseous ampulla of both lateral and anterior canals and on the prominence of the posterior canals, by means of a silver electric thermode (Mancinelli, 1975) The caloric stimulus was controlled in order to induce in the heated ampulla an increase in temperature of maximum 2°C This temperature difference always induced eye nystagmic beating on the plane of the stimulated canal (Flourens 1842)

In the absence of spontaneous eye nystagmus, the animals were curarized with 1–2 mg of Tubocurarine i p and artificially ventilated Under surgical anaesthesia, following appropriate craniotomy, the temporoparietal lobes and the occipital poles were removed by suction, in order to expose the dorsal surface of posterior thalamus, the IIIrd ventricle and the *corpora quadrigemina* which were carefully protected with warm mineral oil maintained at a constant temperature of 37 5°C The wound edges and the pressure points of

the stereotaxic apparatus were repeatedly infiltrated with Xylocaine In order to record unitary discharges, a tungsten microelectrode (tip diameter 1–4 μm, resistance 600–1 000 KΩ) was driven by means of a micromanipulator into the medial geniculate bodies and in few experiments, in the *brachia colliculi inferioris*, in the inferior colliculi and in the VIIIth vestibular nerve The recording micro electrode was connected through a P5 Grass preamplifier to a Tektronix 565 dual-beam oscilloscope and to a Hewlett-Packard 3960 instrumentation recorder

In order to evaluate the possible spread of thermal stimulus applied to a single semicircular canal, a thermocouple was placed in the neighbouring ampulla negligible changes in temperature (0 1–0 5°C) were recorded In some experiments, tests were performed to ascertain whether this temperature change was capable of modifying the resting activity of VIIIth nerve fibres Thus, a primary vestibular afferent responsive to a single canal was identified Thermal stimulations greater than the ones usually used, applied to the other ampullae, did not modify the resting activity of this vestibular fibre In this way, it was possible to rule out thermal stimulus spread to additional semicircular canals

Click and diapason (440 Hz) stimulations at variable intensity were used to study the binaural acoustic influences on MGB units Click stimuli were obtained by applying 0 1 msec pulses delivered from a 160–161–162 Tektronix waveform generator appropriately amplified and connected to a loudspeaker This latter stimulus was preferred because of the similarity between its components and the ones commonly occurring in most natural sounds In addition clicks produce widespread activity throughout the acoustic path way and higher auditory neurons while pure tones do not (Aitkin & Dunlop 1968 1969)

The thermal stimulation of the single semi circular canals could be performed in the absence of auditory stimuli as the animals were isolated acoustically The recorded sites in the

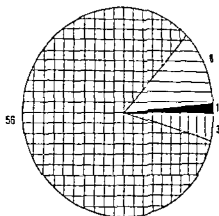


Fig 2 The chart illustrates the responsiveness of the 68 units recorded in MGB Black area non responsive units Vertical bars acoustically affected units Horizontal bars units driven only by vestibular inputs Cross-hatched area units influenced by both types of stimulation

brain stem were marked by discrete electrolytic lesions At the conclusion of each experiment, the animal was given a lethal dose of anaesthetic, the brain stem was removed fixed in Carnoy solution, embedded in paraffin, sectioned (12 μm) serially and stained according to the Nissl method for the localization of the microelectrode tip (Fig 1)

RESULTS

In the present experiments, 90 units have been recorded 68 were localized in the medial geniculate bodies, 16 in the *colliculi inferioris* and 6 in the *brachia colliculi inferioris*

Regarding the units recorded within the MGB, their histological localization was determined following the anatomical classification of the guinea pig thalamus by Gerebtzoff (1950) Two units were recorded from the *nucleus pronucleus dorsalis*, 51 from the *nucleus pro fundus* and 15 from the *nucleus ventralis* However, the MGB has been dealt with as one entity since response types were not characteristically unique for each of these nuclei (Liedgren et al , 1976)

In resting conditions, 23 out of 68 MGB units (33 8%) exhibited tonic activity with discharge frequency ranging from 10 to 25/sec

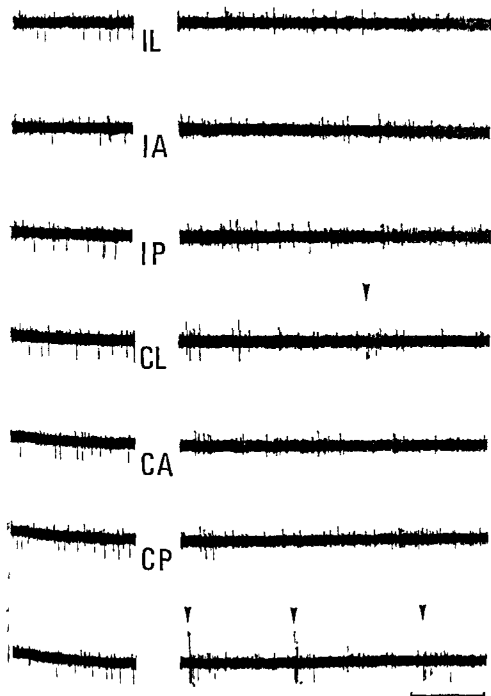


Fig 3 Records taken from left medial geniculate body following vestibular and acoustic stimulations Unit no 64 The left column shows the unitary discharge in resting conditions the right one during stimulation Note in the first six strips total *blocking* of unitary discharge following thermal stimulation of the six single semicircular canals In the last strip the same unit was tested with click (*arrows*) stimuli Note in CL a burst of activity induced by click during vestibular inhibition Abbreviations *IL* ipsilateral lateral *IA* ipsilateral anterior *IP* ipsilateral posterior *CL* contralateral lateral *CA* contralateral anterior *CP* contralateral posterior, canals Calibration 25 mm/sec

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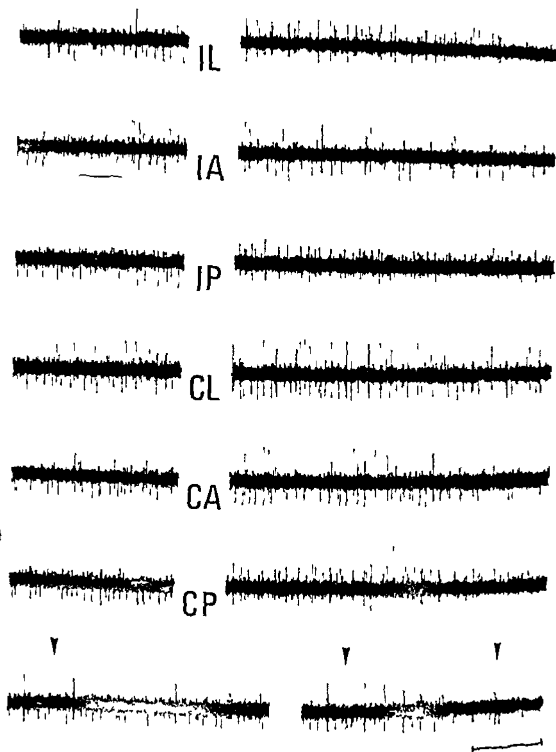


Fig 4 Unitary recordings taken from right medial geniculate body Unit no 59 In the first six strips effects of thermal stimulation applied to the single semicircular canals control on the left responses on the right side Following *IL IP* and *CP* separate stimulations the unitary discharge was inhibited and recruitment of new units was observed A slight decrease in frequency rate is shown [...] *IA CL* and *CA* stimulations were uneffective Ca[...] shown by arrows in the last strip delivered to the s[...] unit enhanced the discharge rate Abbreviations and [...]bration as in Fig 3

ble I *Number of MGB units influenced by rmal stimulation of individual semicircular nals*

...circular canals	Responsive units
	5
	6
	11
	18
	9
	15
al	64

rty four (64 7%) units showed tonic activity th irregular discharge frequency Only one it (1 5%) presented rhythmic discharge with ike frequency of 30/sec The unitary polarity is mostly negative–positive, although at ...es some positive–negative spikes were re... rded In order to minimize possible changes firing rate due to the variable excitability the MGB neurons (Wepsic, 1966), the ef... cts of the stimulation were compared with e immediately preceding unitary distribu... on Furthermore, the unitary waveform and ...ltage were repeatedly observed at high ...eed sweep, throughout the whole period of ...sting in order to exclude possible frequency ...odifications due to changes in electrode ...sition and to recording of other cells

Sixty four of the 68 recorded units were af... cted by vestibular stimulation and 56 of ...ese units also responded to acoustic stimuli, ...ree units were driven by the auditory stimu... ation only and one unit was completely un... sponsive (Fig 2)

All the acoustically influenced units, ir... ...spective of their histological localization, ...splayed a brief excitation represented by ...ursts of 8–18 spikes which followed every ...ngle click (Figs 3, 4) Marked variability in ...xcitatory response frequency occurred, in... ...ependently of stimulus intensities (Aitkin et ...l 1966) The same pattern of modification ...as also observed when clicks were applied ...uring vestibular thermal stimulation (Fig 3, ...L)

All the vestibular affected units were re corded from the *nuclei profundus* and *ven tralis* Thermal stimulation of individual semi circular canals of both labyrinths affected 42 units, while 22 were influenced by only one labyrinth (14 contra and 8 ipsilaterally) Most units were driven by more canals, as shown in Table I, from which the convergence of more ampullary inputs on the same unit can be ap preciated Vestibular peripheral stimulation modified the discharge pattern of MGB units independently of the stimulated canal and no preponderance of effectiveness among canals was observed Table II shows the effects in duced by stimulation of ipsi and contralateral single semicircular canals on the activity of the MGB units recorded Modifications of unitary discharge appeared after 2–4 sec from stimulus onset and disappeared when the stimulus was over they consisted of inhibition (or disfacilitation) or excitation (or disinhibi tion) A decrease in discharge rate to 5–15/sec (Fig 4, *IA*) was the most frequently en countered modification, total blocking of uni tary discharge (Fig 3) was also observed The excitatory response consisted of an increase in discharge frequency up to 20–60/sec Occa sionally recruitment of new units could be re corded (Fig 4, *IL IP CP*) and some tonic units could exhibit rhythmic activity following labyrinthine stimulation

From the analysis of 22 units recorded in the inferior colliculus and its *brachium*, 33% ex citatory effects and 15% inhibitory influences were obtained, following single canal stimula tions Bilaterality of labyrinthine projections was also encountered at these levels (Fig 5)

DISCUSSION

In previous works, convergence of vestibular inputs on several brain stem units localized in the lateral vestibular nucleus, paramedian pontine reticular formation oculomotor nucleus, nystagmogenic cortical area has been demonstrated in the guinea pig (Desole & Pal lestrini, 1969, Manni & Desole, 1966, Palle-

Table II *Pattern of response of MGB units to thermal stimulation of single semicircular canals*

Canals	Excitation	Inhibition	No influence	Total influenced units
IL	10	21	33	64
IA	10	12	42	64
IP	11	20	33	64
CL	13	21	30	64
CA	16	16	32	64
CP	11	24	29	64
Totals	71	114	199	

strini & Desole, 1974, Petrosini et al , 1975, Troiani et al , 1976) The purpose of the present work was to analyse, in single units localized in the medial geniculate body, convergence of labyrinthine inputs from single semicircular canals and to investigate acoustical and vestibular convergence Thermal stimulation applied to individual osseous ampullae has been proven to be selective, as demonstrated in the Methods, and advantageous for the study of afferences from single canals on single neurons An analysis of unitary discharge of MGB neurons in the guinea pig has never been undertaken, notwithstanding the anatomo functional indications of Gerebtzoff (1950) on the arrival on this thalamic nucleus of vestibular fibres on their way to the cortex

According to our results the medial geniculate body is reached by vestibular and acoustic afferences in the territory of the *nuclei ventralis* and *profundus*, while units localized in the *nucleus dorsalis* were not influenced by vestibular stimulation This is in agreement with the classification of Gerebtzoff (1950) who claimed that the *nucleus dorsalis* is reached by acoustic and optic fibres only The patterns of unitary modification recorded in MGB following vestibular stimulation can be summarized as follows (i) 94% of the units analysed were influenced by vestibular inputs, of which (ii) 62% responded to separate stimulation of both labyrinths and 32% to only one (mainly contralaterally), (iii) 72% of the recorded units were affected by inflows ar... from several canals and 22% from one ca... only, (iv) the MGB unitary responses f... ing stimulation of individual semicircular ca... nals of the ipsilateral labyrinth were qual... wise to those obtained by stimulating the contralateral single ampullae

On this basis it seems reasonable to assu... a wide vestibular projection on MGB ... result is in agreement with recent electro... physiological studies in cat and prim... which have demonstrated that vestibulo... thalamic projections are directed particul... to the ventro basal complex and to the po... terior nuclear group, including MGB (... et al , 1972, Deecke et al , 1974, Liedgren & Schwarz 1976, Liedgren et al 1976 Ra... mond et al , 1974, Sans et al , 1970 Weps... 1966) The responsiveness of MGB neur... both labyrinths, indicates a bilateral arr... of vestibular inflow, this result gains ... from Odkvist et al (1973) who demonstra... on the bilateral cortical representation of ... vestibular nerve in the guinea pig The d... charge modifications induced on the MGB unit by inputs from single ampullae ... dicate the existence of convergence of ... lar volleys on these neurons Therefore MGB units under the influence of inputs f... both vertical and horizontal canals this th... lamic nucleus can be assumed to be a proj... tion structure to other subcortical and ... cal centres of information on head move... and position (Manni, 1952)

The MGB unitary discharge pattern ... modified by vestibular peripheral stimu... with either excitation or inhibition the L... being the most frequently observed C... sidering that the same MGB units were ... fluenced by both acoustic and vestibular ... puts, an attempt to explain the vestibula... hibitory response leads us to suppose ... hibitory action of Golgi II interneurons (M... rossy & Kiss 1976*a* 1976*b*), as demons... with intracellular recordings by other ... following acoustic stimulation (Aitkin & ... lop, 1968, 1969, Etholm, 1976) F...

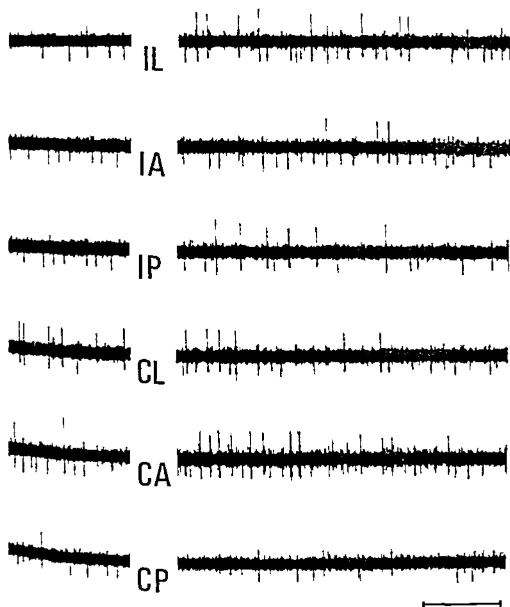


Fig. 5. Unit no. 5 recorded from left *brachium coll. cul. inferioris*. The left column depicts the resting unitary discharge, the right one the response to thermal stimulation of the six single semicircular canals. Four canal (*IL, IA, CL, CA*) stimulation increased the discharge rate while both posterior canals were uneffective. Abbreviations and calibration as in Fig. 3.

units recorded in the inferior colliculus and its brachium were driven by thermal labyrinthine stimulation mostly with an increase in discharge frequency. Consequently the inhibitory effects observed in MGB ought to be generated by intrinsic circuits.

MGB unitary responses to our acoustic stimulation, always given binaurally, consisted in agreement with previous investigations (Aitkin et al. 1966, Aitkin & Dunlop 1968) of excitatory effects. Convergence of acoustic and vestibular inputs on the same MGB unit is thus claimed.

Speculations on the possible role of this

thalamic nucleus regarding the mechanism of head orientation need more detailed physiological studies and will be the aim of further investigations

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ZUSAMMENFASSUNG

In den curarisierten Meerschweinchen wurden 68 Neurone des Corpus Geniculatum Mediale (CGM) mit vestibularen und akustischen Reizen untersucht Die einzelnen Bogengange wurden termisch gereizt Konvergenz der vestibularen und akustischen Afferenzen an denselben Einheiten des CGM wurden registriert Der Reizung der Bogengange folgend, wurden Hemmung und Aktivierung der Einheiten aufgezeichnet, wobei die Hemmung vorherrschend war, wahrend bei akustischer Reizung („Klick") Salven neuronaler Aktivität verzeichnet wurden Es wird die Beteiligung des CGM in die vestibulo-akustische Integration postuliert

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ACTOMYOSIN ATPase ACTIVITY OF HUMAN LARYNGEAL MUSCLES

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Abstract The muscles from seven human larynxes removed by laryngectomy have been examined for actomyosin ATPase by histochemical methods The various muscles contained a mixture of ATPase low (type I) and ATPase high (type II) muscle fibres The thyreoarytenoid muscle had the highest proportion of type II fibres (65%) and the posterior cricoarytenoid muscle had the highest proportion of type I fibres (67%) The other laryngeal muscles had intermediate valves All human laryngeal muscles had a higher percentage of type I fibres than the corresponding muscles in animals so far examined a finding which may be related to the development of speech

There is a close correlation between the contraction speed of a muscle and its activity of actomyosin ATPase (Bárany, 1967) In most mammalian muscles two main types of muscle fibres can be identified, one with low actomyosin ATPase activity (type I) and the other with high actomyosin ATPase activity (type II) (Guth & Samaha, 1969, Brooke & Kaiser, 1970)

In a combined physiological–histochemical study in the cat it has been shown that the motor units consisting of type II muscle fibres have a fast contraction speed and those consisting of type I fibres have a low contraction speed (Burke et al , 1973)

The contraction speed of the laryngeal muscles has been the subject of interest to many investigators While the thyreoarytenoid muscle in most species appears to be a fast muscle (Mårtensson & Skoglund 1964, Hirose et al , 1969, Hast, 1969) or even, as in the rabbit, an extremely fast muscle (Hall-Craggs 1968) there appear to be certain species differences with other laryngeal muscles The posterior cricoarytenoid muscle is fast in the cat (Hirose et al , 1969), but slow in the dog (Mårtensson & Skoglund, 1964, Hast 1967) The cricothyroid muscle is fast in the squirrel monkey (Hast, 1969) and in the rabbit (Hall-Craggs, 1968), but slow in the gibbon in the macaque (Hast, 1969), in the dog (Mårtensson & Skoglund, 1964, Hast, 1966) and in the cat (Hirose et al , 1969)

There is also considerable interspecies variation in the proportion of type I and type II fibres in the various laryngeal muscles in animal species examined so far (Asmussen & Wohlrab, 1972, Sahgal & Hast, 1974)

With the development of speech it is likely that the laryngeal muscles in man have evolved differently from those of the common laboratory animals The aim of the present investigation was to see to what extent this functional difference is reflected in the proportion of type I and type II muscle fibres

MATERIALS AND METHODS

Intrinsic laryngeal muscles were dissected out from the healthy side of human larynxes obtained during laryngectomy on 7 males wi

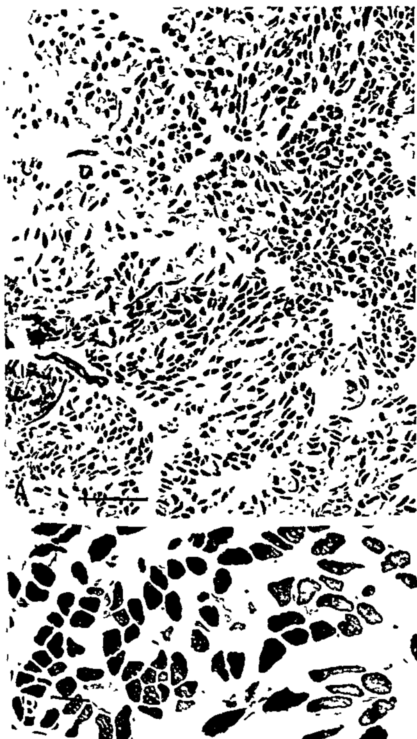


Fig 1A B Human thyreoarytenoid muscle Patient treated with 60 Gy (=6000 rad) cobalt 3 months pre operatively 16 μm section incubated for ATPase at pH 9 4 after preincubation in buffer pH 10 3 with 50 mM $CaCl_2$ and buffer pH 4 6 Type I muscle fibres light Type II muscle fibres dark Scale 500 μm (A) and 100 μm (B)

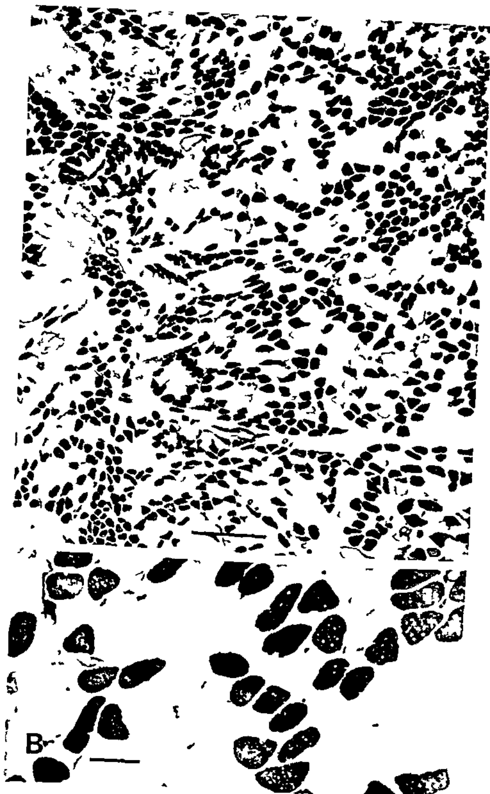


Fig 2A B Human cricothyroid *muscle* Patient treated with conventional radiation (300 kV) 4 00 R total skin dose and 180 mg Bleomycin 1 year preoperatively In cubation and preincubation conditions as in Fig 1 ᶠ 500 μm (A) and 100 μm (B)



Fig 3A B Human interarytenoid muscle Primary laryngectomy Incubation and preincubation conditions as in Fig 1 Scale 500 μm (A) and 100 μm (B)

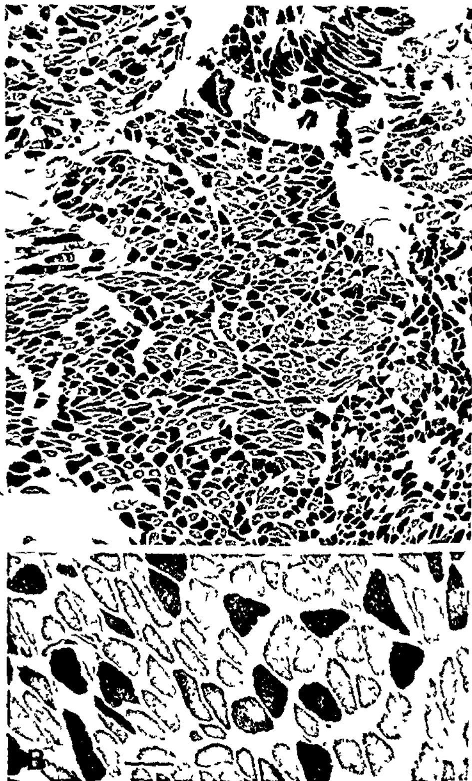


Fig 4A B Human lateral cricoarytenoid muscle from patient treated with conventional *radiation (300 kV)* 4 200 R total skin dose and 180 mg Bleomycin 8 months *pre*operatively Conditions otherwise as in Fig 1 ex the alkaline buffer contained 100 mM $CaCl_2$ instead

unilateral laryngeal carcinoma Two patients had a primary laryngectomy The remaining 5 patients had been treated with a combination of 180 mg Bleomycin and X-rays, (4200 R total skin dose) 3 months to 6 years prior to the laryngectomy The muscles from the primary laryngectomies served as a control to rule out possible effects of the cytostatic and X ray treatment on the ATPase activity of the muscles

Each muscle was divided in two to eight pieces according to a standardized scheme to ensure proper freezing and to detect possible regional variations in muscle fibre types The pieces were mounted in Cryoform (Damon/IEC division) on brass chucks, oriented for cross sectioning and frozen in liquid nitrogen The muscles were stored at −85°C until further processing Serial sections, 16 μm thick were cut on a Dittes Duspiva cryostat and incubated for actomyosin ATPase according to Guth & Samaha (1970) Some sections were sequentially incubated in alkaline buffer (pH 10 1–10 3) containing $CaCl_2$ (10–100 mM) and acid buffer (pH 4 2–4 6) to improve separation of fibre types (Grønnerød et al , in prep) Counting of fibres was performed in a Zeiss standard microscope equipped with a drawing apparatus projecting onto the table

Areas with good staining and proper transverse sections of muscle fibres were selected for counting The code on the slide was not known to the person who did the counting, thus preventing possible biased selection of areas

RESULTS

In all muscles there was a mixture of type I and type II muscle fibres No fundamental difference in proportion or distribution of the two types could be detected between the muscles obtained by primary laryngectomy and the corresponding muscles which had been subjected to cytostatic and X ray treatment preoperatively The fibre diameter of the muscles removed by primary laryngectomy appeared slightly larger than that of the corresponding muscles in the rest of the material A possible X ray induced atrophy of muscle fibres can therefore not be ruled out

The various laryngeal muscles showed marked difference in muscle fibre composition Examples are shown in Fig 1*A*, *B* (thyroarytenoid), in Fig 2*A*, *B* (circothyroid), in Fig 3*A* *B* (interarytenoid) and in Fig 4*A*, *B* (lateral cricoarytenoid) There was a great variation in the diameter of both the type I and the type II muscle fibres within each of the muscles Generally the fibre diameters of the thyreoarytenoid and of the interarytenoid muscles were smaller than those of the other laryngeal muscles in the same person

In transverse sections of the thyreoarytenoid muscle it was not possible to find any sharp delineation between the vocalis part and the lateral part of the muscle The muscle fibres in the vocalis part were thinner than the fibres in the lateral part, but we could not find any difference in the proportion of type I and type II fibres in the two parts Nor could we find any regional variation of fibre types in any of the other laryngeal muscles apart from type II fibres in the two parts Nor could we oid muscle of one patient (Fig 5 A, B) This patient had received X ray treatment and Bleomycin 1 year preoperatively but none of the other muscles of this patient showed any abnormality of this kind On the other hand, type ' grouping has been found in a posterior cricoarytenoid muscle obtained at autopsy from a person without any history of laryngeal disease (unpubl observation) An average of 1 126 fibres in each muscle was counted Table I shows the percentage of the two fibre types in each muscle The thyreoarytenoid muscle had the highest percentage of type II fibres (65±11 6%), and the posterior cricoarytenoid muscle had the highest percentage of type I muscle fibres (67±8 6%) The other muscles had intermediate values

Acid preincubation (pH 4 2) gave activation of type I fibres and inactivation of type II fibres (acid reversal) in all the laryngeal mus



F g 5A B Type group ng of muscle f bres n human posterior cricoaryteno d muscle Same p ent and h stochem cal treatment as in F g ?

Table I

Muscle	% type I ±S D	% type II ±S D
Thyreoarytenoid	35±11 6	65±11 6 n=7
Lateral cricoarytenoid	40± 5 2	60± 5 2 n=6
Interarytenoid	46± 8 7	54± 8 7 n=5
Cricothyroid	47± 6 1	53± 6 1 n=7
Posterior cricoarytenoid	67± 8 6	33± 8 6 n 7

cles Acid preincubation at different pH values was attempted in order to identify subgroups of the type II fibres (Brooke & Kaiser, 1974) Inactivation of the ATPase activity of most type II fibres took place gradually in the range pH 4 6–4 2 It was therefore not possible to tell with certainty which of the muscle fibres corresponded to the different subgroups of the type II fibres

DISCUSSION

The present results show that there is a great variation from person to person in the proportion of type I and type II fibres in the various laryngeal muscles In this respect the laryngeal muscles resemble ordinary human skeletal muscles, which also show considerable interpersonal variation (Johnson et al , 1973)

The high percentage of type II muscle fibres in the thyreoarytenoid muscle is surprising considering that 70–80% of the fibres in the medial two-thirds of this muscle have been found to have multiple motor end plates (Rossi & Cortesina 1965) In lower vertebrates multiply innervated muscle fibres have been shown to be of slow non twitch type (Kuffler & Vaughan Williams, 1953) In the cat, multiply innervated muscle fibres have been found in the external eye muscles (Hess & Pilar, 1963) and in the middle ear muscles (Fernand & Hess 1969) From existing evidence they seem to be of both slow non twitch and of slow twitch type (Hess & Pilar, 1963, Hess, 1970 Bach y Rita & Ito, 1966, Lennerstrand 1974 Browne, 1976)

According to Hall Craggs (1968) the thyreoarytenoid muscle of the rabbit is extremely fast, and although interspecies variations may exist, it is still hard to account for the high percentage of multiply innervated muscle fibres in the thyreoarytenoid muscle More research on this point is necessary to clarify the matter

It is not possible from the proportion of type I and type II fibres alone to tell whether a muscle as a whole is fast or slow However, by comparison with other muscles, e g the cat tensor tympani which is a fast muscle (Teig, 1972) and which consists of 40% type I and 60% type II muscle fibres (Teig & Dahl, 1972), it seems probable that the thyreoarytenoid muscle in man is a fast muscle

In the rabbit Asmussen & Wohlrab (1972) found the thyreoarytenoid muscle to consist exclusively of type II fibres, while the cricothyreoid muscle consisted of 65% type II and 35% type I fibres, and the posterior cricoarytenoid muscle of 60% type II and 40% type I fibres Comparable ATPase activity was found in muscle homogenates from rabbit cricothyroid and thyreoarytenoid muscles (Syrovy & Gutmann, 1971) In the rhesus monkey Sahgal & Hast (1974) found 81% type II and 19% type I fibres in the thyreoarytenoid, 70% type II and 30% type I in the cricothyroid and 88% type II and 12% type I in the lateral cricoarytenoid muscle

The present results show that the percentage of type I muscle fibres in all the laryngeal muscles in man is much higher than in the animals so far examined This must be kept in mind when data from man are related to data from experimental animals Since type I muscle fibres at least in ordinary skeletal muscles are slow and more resistant to fatigue than type II muscle fibres (Burke et al , 1973) the high percentage of type I muscle fibres in human laryngeal muscles might be related to the development of speech with demand for frequent and longlasting activity of these muscles

The muscles with the highest proportion of type II muscle fibres, the thyreoarytenoid,

the lateral cricoarytenoid and the interarytenoid, all contribute to the sphincter function of the larynx The need for fast action in this protective mechanism which is the phylogenetically oldest and most important function of the larynx (Negus, 1949), is evident The cricothyroid muscle, whose main action is to produce tension in the vocal cords, and which is the most important muscle for the variation of pitch (Koyama et al , 1971), has a higher proportion of type I muscle fibres Again the higher proportion of type I fibres in this muscle in man might reflect a need for endurance and fatigue resistance in voice production The sole abductor of the larynx, the posterior cricoarytenoid, which is active during the inspiratory phase of respiration (Faaborg-Andersen, 1957) has a higher proportion of oxidative enzymes than the other laryngeal muscles (Hefter, 1967, Hanson & Lotz, 1973) and was found to have the highest proportion of type I fibres in the present investigation This constellation is probably related to the continuously rhythmic action of this muscle during respiration

When the recurrent nerve of experimental animals is stimulated with supramaximal stimulus amplitude, a low stimulus frequency induces abduction of the vocal cords, whereas higher stimulus frequency leads to an increasing adduction (Ohyama et al , 1972) This has *also been observed in the human larynx* (Kotby & Haugen, 1970)

The present findings offer a possible explanation of this The slow muscle fibres are brought into tetanic contraction and thus exercise their full force at a low stimulus frequency while the fast fibres need a considerably higher stimulus frequency to reach full tetanic tension A low stimulus frequency would therefore be more effective to the posterior cricoarytenoid muscle than to the other muscles, and a high stimulus frequency, giving tetanic contraction of all muscle fibres, would lead to adduction since there is an overweight of adductor muscles in the larynx (Pressman & Kelemen, 1955)

"Grouping" of type I and type II fi[illegible] muscle is assumed to be a result of [illegible] muscular degeneration where de[illegible] muscle fibres are reinnervated by [illegible] sprouting from adjacent intact motor [illegible] (e g Edstrom & Kugelberg 1969) Lary[illegible] paresis with impaired abductor function is [illegible]latively common in horses (roarers") [illegible] (1972) found type "grouping" of fibres in [illegible] posterior cricoarytenoid muscle in a few [illegible]parently normal horses This was interp[illegible] as indicating a tendency to spontaneous [illegible]generation of this muscle, and it was [illegible]gested that the affected horses later [illegible] have become clinical "roarers'

The type "grouping ' in the posterior cri[illegible]arytenoid muscle in one of our patients [illegible] an unexpected finding since the patient [illegible] no clinical signs of laryngeal paresis [illegible] posterior cricoarytenoid muscle is [illegible] more exposed to mechanical trauma such [illegible] endoscopy or even possibly swallowing of [illegible] lumps of food, and this might be an expla[illegible]tion for the selective affection of this mus[illegible] in our patient The finding also lends supp[illegible] to the musch disputed Semons "law [illegible] the greater vulnerability of this sole [illegible] muscle of the larynx

ZUSAMMENFASSUNG

Die Muskeln sieben Kehlköpfe, die durch [illegible] entfernt worden waren *wurden auf Actomyosin* [illegible] mit histochemischen Methoden untersucht [illegible] denen Muskeln enthielten ein Gemisch von ATP[illegible] schwachen (Typ I) und ATPase starken (Typ II [illegible]fasern Der grösste Anteil Typ II Fasern (6[illegible]) [illegible] Muskulus thyreoarytenoideus der grösste Anteil T[illegible] Fasern (67%) in Muskulus cricoarytenoideus [illegible] Die ubrigen laryngealen Muskeln zeigten dazwi[illegible]gende Werte Die laryngealen Muskeln des Mens[illegible] zeigten einen höheren Anteil von Typ I Fasern a[illegible] entsprechenden Muskeln der Tiere ein Befund der [illegible] der Entwicklung der Sprache möglicherweise zu[illegible] hängt

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QUANTITATION OF HUMAN GRANULOCYTE PROTEASE INHIBITORS IN NON PURULENT BRONCHIAL LAVAGE FLUIDS

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Abstract The predominant inhibitors of granulocyte proteases in plasma (α_1 antitrypsin α_1 antichymotrypsin and α_2-macroglobulin) were quantitated in unconcentrated bronchial lavage fluids obtained from non infected individuals together with the acid stable low molecular weight inhibitor with activity against granulocyte elastolytic and chymotrypsin like enzymes This latter inhibitor accounted for about 90% of the total molar concentration of granulocyte protease inhibitors in the bronchial lavage fluids The remaining 10% consisted mostly of α_1 antitrypsin and α_1 antichymotrypsin About 85% of the bronchial inhibitor was in a free form with preserved enzyme reactivity The remaining 15% of the immunoreactive bronchial inhibitor exhibited a molecular size indicating complexation with enzymes The major portion of α_1 antitrypsin and α_1 antichymotrypsin showed electrophoretic mobilities and molecular sizes similar to the native proteins but had no enzyme reactivity

Normal bronchial secretion is difficult to obtain from man, hence most earlier studies have been performed on purulent expectorates (Ryley & Brogan, 1973 Lebas et al , 1976) Specific immunochemical techniques demonstrate the presence of most serum proteins in such secretions (Biserte et al 1963, Dennis et al , 1964) and albumin and IgA are thought to be the predominant plasma proteins (Anzai et al , 1963, Ibayashi et al , 1963 Dennis et al , 1964, Masson et al , 1965) The major inhibitors of proteolytic enzymes in plasma such as α_1-antitrypsin, α_1 antichymotrypsin and α_2 macroglobulin, have also been identified in bronchial secretions (Ryley & Brogan 1973 Lebas et al , 1976) α_1-Antitrypsin was shown to be the inhibitor with the highest molar con centration as in plasma (Hochstrasser et al 1972, Laurell & Jeppsson 1975) This in hibitor inactivates the elastolytic, chymotryp sin like, and collagenolytic enzymes of granu locytes (Ohlsson & Olsson 1973 1974a 1974*b*, Ohlsson, 1975, Venge et al 19 (Table I) α_2 Macroglobulin also inhibits all known granulocyte proteolytic enzy (Ohlsson & Olsson, 1973, 1974*a* 1974*b* Ohl son, 1975, Venge et al , 1975) but α_1 chymotrypsin is specific for the chymotryp like granulocyte proteases (Ohlsson & Ak son, 1976) (Table I)

Bronchial secretions also contain an a stable, low molecular weight inhibitor of p *teolytic enzymes* (Hochstrasser et al 19 Ohlsson et al , 1977) which is a strong inh of elastolytic (Ohlsson & Tegner, 1976) as as chymotrypsin like (Tegner et al , 197 zymes from granulocytes but not the co genolytic enzyme (Ohlsson & Tegner 1 (Table I)

Granulocyte elastase was recently show a pilot study to rapidly destroy the mucoc

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le 1 *Inhibition specificity of the major protease inhibitors in human bronchial mucus*

ame	Mol wt (D)	Inhibition of granulocyte proteases: Elastase	Collagenase	Chymotrypsin like cationic proteins
Antitrypsin	55 000	+	+	(+)
Macroglobulin	725 000	+	+	+
it chymotrypsin	69 000			+
onchial mucus	10 500	+		+
b I				

tivity of respiratory epithelium in vitro (Tegr, 1977) The molar concentration of the inhibitors in bronchial lavage fluid may correlate ith the relative importance of each inhibitor the protection of the ciliated columnar epielium of the respiratory tract from granulo- te proteases The purpose of the present in- stigation was to quantitate the predominant hibitors of granulocyte proteases in non- fected bronchial lavage fluids

MATERIAL AND METHODS

ronchial lavage fluid was aspirated during onchoscopy of 51 patients (35 males and 16 males) Their ages ranged from 35 to 78 ars The indications for bronchoscopy were ually prior hemoptysis, or suspected or own malignancy The patients were afebrile d exhibited no signs of respiratory tract in ction or other lung disease at the time of onchoscopy

Under general anaesthesia a rigid broncho- ope was passed into one of the main stem onchi without prior endotracheal intubation ml of sterile saline was instilled under sterile nditions and gently aspirated to prevent in vertent damage to the mucous membrane lood-contaminated or purulent samples were iscarded

The debris and cells of the lavage fluids ere separated by centrifugation at 3 000 *g* for 5 min within 2–4 hours after collection, and e supernatant was frozen and stored at 20°C until analysis which was performed without prior concentration of the super natant For qualitative studies 0 5 ml of each lavage fluid specimen was pooled and con centrated to about 2 ml in an Amicon cell with a Diaflo UM 2 membrane at 0°C This pool was used to determine the free or complexed in hibitor levels in the samples and to measure the total protease inhibiting capacity of the specimens

Special materials Sephadex G 75 and G 150 were purchased from AB Pharmacia, Uppsala Sweden, and agarose from Miles Seravac, Maidenhead England

Antisera Specific antisera against albumin, α_1 antitrypsin, α_1-antichymotrypsin, and α_2 macroglobulin are available in our laboratory Rabbit antiserum against the low molecular weight inhibitor from bronchial secretion was prepared as previously described (Ohlsson & Tegner, 1976)

Granulocyte proteases Granulocyte elas tase was purified as described (Ohlsson & Ols son, 1974*a*) and chymotrypsin like cationic proteins were obtained from Dr Inge Olsson (Olsson & Venge 1974)

Reference proteins A human serum pool of blood donors was used as a reference The concentration of albumin was 46 g/l, α_1-anti trypsin 1 35 g/l, antichymotrypsin 0 4 g/l and α_2 macroglobulin 2 0 g/l

Molecular weights The following molecular weights were used for the calculations of molar concentrations albumin 66 500 (Rothschild et al , 1972) α_1 antitrypsin 55 000 (Laurell & Jeppsson, 1975), antichymotrypsin 69 000

Table II *Frequency and average concentrations of detectable quantities of selected pro... bronchial lavage fluid samples of 51 patients*

Protein	*n*		Mg/litre Mean ± S D	Nmole/litre Mean ± S D
Albumin	51	100%	116 0±146 4	1 744±2 200
α_1-Antitrypsin	43	84%	5 5± 6 8	100± 123
Antichymotrypsin	30	59%	2 6± 2 4	38± 34
α_2 Macroglobulin	21	41%	9 5± 10 6	13± 14
Bronch protease inhibitor	39	76%	15 0± 11 5	1 427±1 095

(Laurell & Jeppsson, 1975), α_2-macroglobulin 725000 (Laurell & Jeppsson, 1975), and the bronchial protease inhibitor 10500 (Ohlsson et al , 1977)

Immunochemical techniques

Albumin and the plasma protease inhibitors were measured by electroimmuno assay (Laurell, 1972) The single radial immunodiffusion technique of Mancini et al (1965) was used for quantitation of the bronchial inhibitor using purified inhibitor as a standard Crossed immunoelectrophoresis was performed according to Ganrot (1972)

Gel filtration

Gel filtration of 0 5 ml of the concentrated pool of lavage fluids was performed on a Sephadex G-75 column (0 6×100 cm) equilibrated with 0 04 M Tris-HCl buffer, pH 7 6, containing 0 2 M NaCl A constant flow of 8 ml per hour was maintained and 0 5 ml fractions were collected On the same column and under identical conditions the elution volumes were determined for Trasylol (M_r 7000), cytochrome C (M_r 12500), myoglobin (M_r 17800) chymotrypsinogen A (M_r 25000) and egg albumin (M_r 45000) Gel filtration was also performed on a Sephadex G 150 column (1 5×100 cm) equilibrated with the same buffer This column was calibrated with myoglobin (M_r 17800), egg albumin (M_r 45000) bovine albumin (M_r 69000), haptoglobin type 1 1 (M_r 98000), and aldolase (M_r 158000) A linear function resulted when the logarithms of their molecular weights were plotted against their elution volumes The fractions eluted after gel filtration of pooled concentrated lavage fluids were analyzed for immunoreactive α_1-antitrypsin, antichymotrypsin, and the bronchial inhibitor

Reaction mixtures of bronchial lavage f... and granulocyte elastase or chymotrypsin ... cationic proteins corresponding to about ... and 100% saturation of the protease inh... capacity of the lavage fluids were incub... for 10 min at 27°C before analysis by cross... immunoelectrophoresis or gel filtration

RESULTS

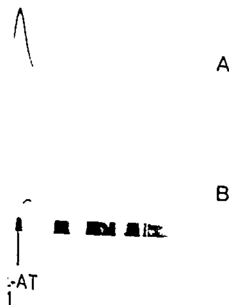
About 1 to 2 ml of bronchial lavage fluid ... aspirated from each patient The fluid ... peared colourless and the debris separa... centrifugation was scanty

Quantitation of albumin α_1-antitrypsin, α_1-antichymotrypsin, α_2-macroglobulin and bronchial inhibitor (Table II)

Albumin was detected in all samples ... mean concentration of 116 mg/l Some ...

Table III *Molar ratios of protease inh... to albumin in normal serum and br... lavage fluids using mean concentra... each group*

Protein	Normal serum	Br... fl...
α_1 Antitrypsin / Albumin	0 035	[illegible]
Antichymotrypsin / Albumin	0 008	[illegible]
α_2-Macroglobulin / Albumin	0 044	[illegible]



Precipitate patterns obtained on crossed immuno-
horesis of human serum (A) and bronchial lavage
) using an antiserum against α_1 antitrypsin Screen
horesis of human serum below for reference

l measurable quantities of one or more protease inhibitors α_1-Antitrypsin was rable in 43 of the 51 samples (84%) with n value of 5 5 mg/l α_1-Antichymotrypsin ound in 30 specimens of the 51 analysed) The mean value was 2 6 mg/l The high ular weight plasma protease inhibitor α_2-oglobulin was present in 21 of the 51 sub- (41%) with a mean value of 9 5 mg/l The limits of measurability of these plasma ins was about 1 mg/l The bronchial in- r was detected in 39 of 51 lavage fluids sed (76%) The mean value of these 39 les was 15 0 mg/l (lower limit about l)

e ratios of inhibitor/albumin was cal- ed using the mean values (Table III) e values for bronchial lavage fluids were ared with corresponding quotients for al human serum The bronchial inhibitor concentration in plasma was below the threshold of detectability with the technique utilized

Homogeneity of α_1-antitrypsin α_1-antichymotrypsin and the bronchial inhibitor in bronchial lavage fluids

Crossed immunoelectrophoresis of a concentrated pool of bronchial lavage fluids and concentrated individual specimens with antiserum against α_1-antitrypsin showed one precipitate peak (Fig 1) This α_1-antitrypsin component showed a slightly retarded electrophoretic mobility compared with native α_1 antitrypsin The addition of different amounts of granulocyte elastase to the lavage fluids before immunoelectrophoresis did not change this pattern

Corresponding analyses with antiserum against α_1 antichymotrypsin of the same bronchial lavage fluids showed one α_1-antichymo-

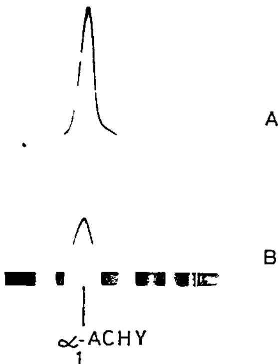


Fig 2 Precipitate patterns obtained on crossed immunoelectrophoresis of human serum (A) and bronchial lavage fluid (B) using an antiserum against α_1 antichymotrypsin Screen electrophoresis of human serum below for reference

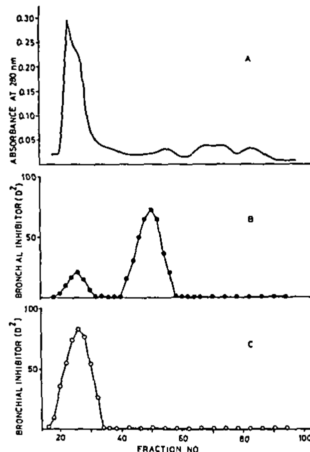


Fig 3 Gel filtration of concentrated bronchial lavage fluid on Sephadex G 75 (A) with the bronchial protease inhibitor localized (B) with the single radial immunodiffusion method of Mancini using antiserum against the bronchial inhibitor In (C) the bronchial inhibitor is localized in concentrated bronchial lavage fluid which had been incubated with granulocyte elastase in excess prior to the gel filtration

trypsin component (Fig 2) This had the electrophoretic mobility of native inhibitor The addition of different amounts of chymotrypsin-like cationic proteins to the lavage fluids before analyses did not change this pattern

On *gel filtration* of bronchial lavage fluids on Sephadex G-150 antitrypsin eluted in a volume corresponding to a molecular size of about 50000 The corresponding value for α_1-antichymotrypsin was 70000 The addition of different amounts of granulocyte elastase or chymotrypsin like cationic proteins respectively before analysis did not change these elution patterns

Unfortunately, the pool of bronchial lavage fluids available was too small to allow detailed studies of the homogeneity of α-macroglobulin

On *gel filtration* of pooled, concentrated bronchial lavage fluid on a calibrated Sephadex G-75 column 85% of the bronchial inhibitor eluted at a volume corresponding to a molecular weight of free inhibitor (10500) (Fig 3) The remaining 15% eluted at a volume corresponding to a molecular weight of about 40–50000 After addition of granulocyte elastase to the lavage fluid all of the inhibitor eluted at a volume corresponding to a complexed molecular weight of 40–50000

DISCUSSION

With the exception of the immunoglobulin IgA, no evidence for a local product ... rum proteins in the lung has been prese... (Asofsky & Thorbecke, 1961, Martinez Te... et al , 1968) In the case of IgA subepith... plasma cells have been shown to contain amounts of this protein by ... technique (Martinez-Tello et al , 1968) F... on results of quantitative immunoe... phoretic studies Ryley & Brogan (1973) ... cently suggested that a selective concen... tion of α_1-antichymotrypsin occurred in ... sputum of patients with chronic bronch... This observation is of interest in view of ... recent finding (Ohlsson & Åkesson 1976) ... α_1-antichymotrypsin is a strong inhibitor ... chymotrypsin like cationic proteins ... granulocytes Furthermore, ... it is a rapidly responding acute phase ... increasing to high concentrations during ... granulocyte phase of inflammatory ... (Aronsen et al , 1972)

Our study utilized bronchial lavage f... from lungs without signs of clinical bronch... No comparison was made of the inhibitor ... centration of the lavage fluids and ... serum specimens Instead, a comparison ... the ratios of albumin to the three pl... tease inhibitors, α_1-antitrypsin, α_1 anti...

ypsin and α_2 macroglobulin in the lavage uids and in normal serum was utilized to emonstrate no selective concentration of ese proteins (Table III)

Because purulent bronchial secretions usually contain free proteolytic activity (Ohlsson & Tegner, 1975) the protease inhibitors are aturated Thus the molecular properties of inhibitors in such secretions may be un-ain The inhibitors may be bound to pro- or partially degraded Those possibil-ies would introduce error in immunochemi- quantitation using free native proteins as The proposed selective concentra- of α_1 antichymotrypsin in the bronchial secretions of bronchitis patients (Ryley & Brogan 1973) will require more detailed studies o be confirmed

The plasma protease inhibitors accounted for only about 10% of the total molar concentration of granulocyte protease inhibitors studied (Table II) The remaining 90% was due to the low molecular weight bronchial inhibitor Gel filtration studies showed that about 85% of the bronchial inhibitor was present as free native inhibitor with the ability to bind granulocyte elastase Thus the quantitative measurements of its concentration in the bronchial lavage fluids are valid The bronchial inhibitor is not measurable in serum by immunodiffusion techniques utilizing the specific antiserum recently produced (Ohlsson & Tegner 1976) Furthermore, its suggested cross reactivity with the inter α trypsin inhibitor (Hochstrasser et al 1973) has not been verified (Ohlsson & Tegner, 1976) A local production of the inhibitor seems likely A recent study (Tegner & Ohlsson, 1977) using an indirect immunoperoxidase technique localized large amounts of the inhibitor in the ciliated columnar epithelium of trachea and in the seromucus glands This certainly strengthens the hypothesis of a local production of the inhibitor in the respiratory tract

A few recent studies (Cohen, 1975, Johnson & Travis, 1976) indicate that α_1 antitrypsin can only bind a protease once Thus α_1 antitrypsin molecules released from their complexes with proteases after transfer of the protease to α_2-macroglobulin, for example, would be expected to demonstrate no further inhibitory capability This α_1 antitrypsin component shows a slightly retarded electrophoretic mobility when compared with native α_1 antitrypsin, an altered mobility similar to the mobilities of α_1 antitrypsin in the samples in this study (Fig 1) The gel filtrations of the lavage fluids after the addition of elastase and chymotrypsin like proteins respectively proved the non reactivity of the major portions of α_1-antitrypsin and α_1-antichymotrypsin present Such non reactive α_1-antitrypsin was found in large amounts in purulent bronchial secretions in an earlier study (Ohlsson & Tegner, 1975) Furthermore, elastase and collagenase in complex with α_1 antitrypsin and as free protease was present in the purulent secretions In the present study the major part of the bronchial inhibitor was free One explanation of the non reactivity of α_1-antitrypsin and α_1 antichymotrypsin might be that they represent the inhibitor partners of previous complexes with granulocyte proteases normally released from granulocytes on the mucous membranes of the respiratory tract The proteases may have been transferred to the bronchial inhibitor or α_2 macroglobulin which is eliminated rapidly This hypothesis would be compatible with the observation that the bronchial inhibitor is partially complexed in the lavage fluids Further studies of the interactions between the bronchial inhibitor and the plasma protease inhibitors and granulocyte proteases will be necessary to clarify the present findings

According to preliminary results granulocyte elastase inhibits the mucociliary activity of tracheobronchial epithelium and causes a digestion of the epithelium with time (Tegner, 1977) This study suggests that a low molecular weight protease inhibitor probably produced locally may be the major component of defence mechanism against proteolytic damage of the respiratory mucosa by granulocyte proteases

ZUSAMMENFASSUNG

Die bedeutendsten Inhibitoren von Granulozyten Proteasen aus dem Plasma (α_1-Antitrypsin, α_1-Antichymo-

mung des Gehaltes von einem niedermolekularen, saure stabilen Proteaseninhibitors statt Die Wirkung des Inhibitors richtet sich gegen elastolytische und chymotrypsin ahnliche Enzyme der Granulozyten Dieser Inhibitor machte etwa 90% der gesamten molaren K

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sachlich aus α_1 Antitrypsin und

Etwa

in freie

des immunreaktiven Inhibitors wiesen ein Molekulargewicht auf, das eine Kopplung zu Enzymen vermuten lässt Der grosste Anteil des α_1-Antitrypsins und α_1-Antichymotrypsins wiesen Elektrophoresmuster und Molekulargewichte auf, die den nativen Proteinen ahneln, jedoch liess sich keine Reaktion mit Enzyme nachweisen

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DESQUAMATION ON TASTE BUDS

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Abstract Taste bud surface cells suffer from a desquamation process that modifies the pores shape and size and the amount of amorphous substance in the pits region We have studied this phenomenon—which probably plays an important functional role—by light electron and scanning microscopy

The taste bud pores and the region of the pit located below, probably play an important role in the first events of the preneural phase of taste Henkin (1969) believes that there actually exists an open and close mechanism in the pore, regulated by biochemical factors

Several of us (Ciges et al , 1976) studied the pore region at rest and after stimulation in order to detect morphological changes by light and electronmicroscopy We used rabbits, one group was killed in the fasting state and another immediately after stimulation with the taste qualities or normal feeding

We were able to detect that the pores in the stimulated animals were more frequently open and contained less amorphous substance than did the control group However, the results were not sufficiently clear to allow any definitive conclusion to be drawn In fact opened pores were evident also in the control group

The tongue has a very unstable mucous membrane that suffers a constant desquamation process and it is subjected to continuous stress by mastication and the various substances with which it comes into contact alcohol tobacco etc The most changeable mechanical and therm stimulus are put c stantly in contact with the tongue surface

According to Ceballos & Ciges (1974) desquamation is a very conspicuous pro in the tongue surface It is an intrinsic , nomenon that is obviously modified by ex trinsic factors In fact they could reali the desquamation is more evident in (omnivorous feeding and various habits) in infants

The morphology and appearance of tongue s surface depend greatly on this pr cess The abnormality of the tongue s that is most obvious in various pathol conditions is mostly a desquamation which has not been adequately studied ably there exists a biological regulating me anism to account for it

We wondered if the physiological tion process could affect the surface of ta buds in the same way as in the rest of tongue Consequently we studied the prob by using light , electron and scanning m scopy in a combined approach in which latter was particularly useful

MATERIAL AND METHOD

Fourteen adult rabbits were used 6 for light microscopy study, 6 for electron scopy and 2 for the scanning study

In each case the animals were killed by



Fig 1 Taste buds occupying the whole thickness of an orthokeratinized epithelium (semithin section toluidine blue staining ×45)

Fig 2 Taste papilla's lateral surfaces with taste buds and desquamated debris in its lumen (semithin section toluidine blue staining ×90)

Fig 3 The pore region with in which are very obvious keratinized cells in its periphery (*arrows*) (EM ×18 000)

capitation The tongues were removed easily in order to obtain the foliate papillae Six of our animals had formed the control group of a previous experiment

For the optical examination we used hematoxylin eosin staining Mallory's method and semithin sections These sections were prepared from the entire surface of the block for electron microscopy and cut in 0 5 or 1 μm sections 1% toluidine blue in borax was used as stain

The specimens for electron microscopy were cut in a prismatic manner keeping a constant orientation reference 2 5% glutaraldehyde and 1% osmium tetroxide were used for fixation with a common buffer system (phosphate solution according to Milloning pH 7 4) Acetone was used for dehydration and the specimens were embedded by Spurr's method

Ultrathin sections and thicker (0 5 or 1 μm) sections were cut on an JKB Ultratome with glass knives at 45° They were examined in a JEM 100B electron microscopy

For scanning electronmicroscopy 1% osmium tetroxide was used for fixation and acetone for dehydration purposes Obtained the specimen under stereoscopic microscope control was evaporated in high vacuum and covered with a thin gold–palladium film and studied by SEM JEOL JSM U_3

RESULTS

In our material studied by light microscopy we can see that the taste buds occupy the whole thickness of the epithelium This epithelium



Fig. 4. Taste papilla's surface in which we can see desquamated cells. Some of them are in the periphery of the pore (SEM, ×2000)



Fig 5. Taste papilla's surface without desqua... Pores of varying size can be seen, together with ... microvilli (SEM, ×6500)

g. 1) is orthokeratinized, and thinner than one on the outer surface. The surface eratinized layers are very evident, though thin. We could see some desquamated cellular elements in the lumen of the circular groove (Figs. 1 and 2). The keratinized layer is interrupted in the taste pores (Figs. 1 and 2).

In the deepest part of the groove, where the serous glands of von Ebner are usually open (Fig. 2), the epithelium is thicker and the keratinization process less evident. Sometimes a para-keratinized covering epithelium was observed, but never a non-keratinized one.

With the electronmicroscope we could observe a typical stratification in the layers of the epithelium surrounding the taste buds.

In the granular layer the keratohyalin granules were evident and desmosomes were conspicuous.

The keratinized layer (Fig. 3) was very ... integrated only by two or three cel... strata—in some specimens, only one devoid... nucleus, organelles or tonofilament ... was a very clear-cut change at the junction ... these layers.

In the micrograph from the ... studied by scanning electronmicroscopy ... can see different features of great interest.

The surface view with scanning perm... us to study both the pores and the desqua... tion process. The shape and size ... are very changeable and some of them ... covered by desquamated cells. In Fig. 4, ... have a view of a papilla, in which we see ... the covering epithelium is a keratinized ... The squamae have an irregular shape ... some of them (see the arrows in the ... form the periphery of the pore. These ... mae correspond to the keratinized cells



Taste pore without desquamated cells It is open void of amorphous substance Some microvilli are (SEM ×10000)



Fig 7 Amorphous substance outside of a pore (*arrows*) (SEM ×16000)

ously by electronmicroscopy Some are occupied by an amorphous substance and others devoid of it In Fig 5 we another aspect of the papillar surface in which there is less desquamation However, can also observe differences between the different pores

some areas the desquamation process not exist and in these circumstances the are usually open and devoid of amorphous substance (Fig 6)

metimes we saw amorphous substance de the pore, an aspect that we can see in 7

DISCUSSION

have been able to observe that desquama phenomena are very evident in the papillae that have taste buds This epithelium is quite similar to that in the remainder of the ortho-keratinized epithelium in the oral cavity, though Squier et al (1976) say that the lateral walls of the papillae are non keratinized and taste buds are present

If we consider the embryological facts—and also according to Farbman (1971)—there is little doubt that the component cells of the taste buds are derived from the epithelium In fact, the epithelium is previous to the buds and the nerves induce it to change in some places in order to form the buds Consequently we must consider that the background of the taste buds is an ortho-keratinized epithelium similar to the one on the outer surface of the papilla

Looking at our light micrographs we can see how the epithelium with taste buds has a nor-

mal stratification process basal layer, prickle cells, granular, keratinized layers However, the taste buds prevent it as foreign bodies

The final event in taste bud development is the formation of the apical pore, which provides direct communication between taste bud cells and the source of their potential stimuli (Farbman 1971) For Farbman no conclusive evidence elucidating the mechanism by which the pore is opened and maintained is yet available For us the mechanism is clear when the transformation of epithelial cells into sensory cells begins, the keratinization process does not exist and the buds prevent the normal stratification process Thus on the surface of every bud, a gap exists in the continuity of the epithelium i e the pore

The desquamation that we have studied on the surface of the taste buds allows us to understand the variability in the size and shape of the pore We have noticed that the surface of the taste epithelium suffers the same desquamation process as is seen in any epithelium Desquamation cells even form the periphery of the pore and when these cells are lost the diameter of the pores becomes enlarged and the microvilli of the sensory cells are freely exposed to the outer ambient This is seen not only in our scanning micrographs but also in the electron micrographs

According to Squier et al (1975–76) a turn over in the oral epithelia exists, on this turn over depends the stratification and evolution of the epithelia but this phenomenon is not the same for the whole no equality of phase exists on the contrary the process is always in a different phase So we could see a very distinct aspect of the taste surface and a very different morphology of the pore according to the phase of the desquamation process

The evolution from the basal layer to the surface is a constant phenomenon When the surface cells on the periphery of the bud are lost, the pore becames wider and it is consequently easy for the amorphous substance to pass out of the pore (Fig 7) because it loses its container

We have come to the conclusion that the desquamation process alters the morphology of the pore and the amount of its amorphous substance content

It is probable that the functional state will differ according to the degree of desquamation The pores seen in Figs 6 and 7 have the microvilli in a more accessible position to be stimulated than those shown in the other micrographs, covered by desquamated cells or occupied by an important amount of amorphous substances

Beidler (1971) suggested a stimulation model based on a mechano-chemical mechanism According to this hypothetical model the microvilli of the sensory cells have cylindrical pores filled with minimally cross linked protein molecules that respond to chemical changes in the environment with a variation of its molecular volume If we consider this stimulation mechanism, contact of the tastants with the surface of the microvilli would be necessary In that case a pore without desquamated cells and amorphous substance would be in the best situations to be stimulated

We could not demonstrate clearly in a previous investigation an open mechanism of the pore with the stimulation Perhaps this mechanism does not exist or else it is only active at a molecular level as Beidler, suggests or perhaps our stimulation method was not appropriate, as it consisted in pouring the test substance into the mouth of the animal with this method there is no real possibility of measuring the effectiveness of the stimulation

Although it was not possible to demonstrate a rapid open mechanism of the pore in direct relation to the stimulus we have been able to demonstrate a slow regulation of the pore size by the desquamation process This process must be regulated by different local and general abstruse factors Guth (1971) observed the extrusion of debris through the taste pore and desquamation of degenerating taste buds from the surface epithelium in denervated

te buds Ceballos & Ciges (1974) saw the
ne phenomenon in normal tongue from
d peoples and others

o we opted for a trophic and direct effect the nerve in the way of the desquamation cess On the other hand Vicente & Ciges 75) studied the taste sense in different lin l pathological tongue conditions and they erved that in the cases with an increase epithelial debris on the surface of the tongue re was a relative hypogousia

ACKNOWLEDGEMENT

want to express our gratitude to Prof H Engstrom the facil ties given to obtain the scanning electron ograph in his laboratory at Uppsala University

ZUSAMMENFASSUNG

Oberflachenzellen der *Geschmacksknospen erleiden* squamat on d e Form und Große der Pore sowie die nge an formloser Substanz verandert Wir haben sen Vorgang—der wahrscheinlich eine wichtige funk nelle Rolle spielt—mit der Rasterelektronenmikro op e sow e mittels der Licht und Elektronenmikro opie studiert

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A SIMPLE DEVICE MEASURING DIFFERENCES IN LEVEL IN THE OESOPHAGUS

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Abstract A device for measuring the difference in level between the pressure transducer and a point of measurement is described It can be used in oesophageal manometry with waterfilled catheters to measure and compensate for superimposed hydrostatic pressure The practical application of the method is illustrated

Accurate measurement of oesophageal pressure using waterfilled catheters has hitherto been possible only with the patient in supine position (Dodds et al , 1976*a*, Dodds, 1976*b*) This is because differences in level between the transducer and the point of measurement in oesophagus result in a hydrostatic pressure added to the oesophageal pressure With the patient in the supine position these variations have been considered negligible

To enable measurement to be made of the difference in level between the pressure transducer and a point of measurement in the oesophagus, a device with a passive catheter has been developed

METHOD AND RESULT

The principle of the passive catheter is shown in Fig 1 The device consists of a U tube, one end of which is filled with water the other being air-filled and freely communicating with the atmosphere The shanks of the passive catheter are polyethylene catheters (PF 100, Portex, Great Britain, inner diameter = 0 9 mm outer diameter = 1 5 mm, length = 1 5 m) connected to a U shaped tube of stainless steel (inner diameter = 0 3 mm outer diameter = 1 0 mm, total length = 30 mm) When measuring oesophageal pressure, the passive catheter is applied to a conventional measurement catheter The transducers of the passive and measurement catheters are kept on the same level The hydrostatic pressure, p, measured with the passive catheter, is directly proportional to the difference in level Δh between the air to-liquid surface and the transducer in accordance with the equation

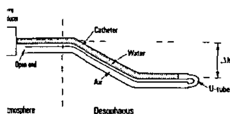
$$p = \Delta h \rho g$$

where ρ is the density of the catheter fluid and g the gravitation constant

Displacement of the air to-liquid interface of the passive catheters owing to variations in temperature and catheter compliance give an error of measurement but with the catheters used this error is negligible The error due to temperature variations is approximately 0 8 mmHg/°C, and that due to compliance approximately 0 1 mmHg/m difference in vertical level

To measure the difference in level in the oesophagus the passive catheter was added to a conventional bundle of three PP 160 catheters with inner diameter 1 0 mm outer diameter

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1 The principle of the passive catheter

ter 1 5 m and with side holes The catheters were connected to transducers (SE Laboratories Feltham, Great Britain) and a three-channel low compliance perfusion pump giving a flow rate of 0 5 ml/min (Ask et al, 1976)

Fig 2 shows examples of intra oesophageal differences in vertical level measured with the passive catheter in a recumbent patient The oesophagus level in relation to the level of the lower oesophageal sphincter (LES) varies in the range 0 to 25 mm Measurements of oesophageal pressures made with stepwise pull through from the stomach to the distal part of the oesophagus in a sitting patient are given in Fig 3 The recordings show pressures measured with the perfused catheter and the same pressures after compensation for differences in level with the aid of the passive catheter

CONCLUSION

The device with a passive catheter makes it possible to measure differences in vertical level between the pressure transducer and the point of measurement in the oesophagus

By subtraction of the pressure measured with the passive catheter from the pressure with the measurement catheter the intra oesophageal pressure in relation to the atmospheric pressure is obtained By this means it is possible to measure the pressure in all body positions and also to measure the divergence of the oesophagus from the direction of the body axis

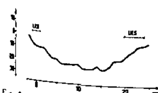


Fig 2 The difference in vertical level (mm) in the oesophagus expressed as a function of the distance (cm) from the lower oesophageal sphincter (*LES*) to a point of measurement in a recumbent patient *UES*, upper oesophageal sphincter

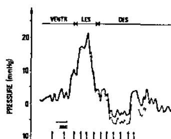


Fig 3 The intra-oesophageal pressure in a sitting patient Each pull through step (1 cm) from the ventricle (*VENTR*) through the lower oesophageal sphincter (*LES*) to the oesophagus (*OES*) is indicated by an arrow The graphs show the measured intra-oesophageal pressure (—) and the same pressure after compensation for differences in level measured with the passive catheter ( )

ZUSAMMENFASSUNG

Eine Vorrichtung wird beschrieben mit der man Niveau unterschiede zwischen einem Druckgeber und einem Meßpunkt feststellen kann Diese kann bei Oesophagus manometrie mit wassergefüllten Kathetern verwendet werden um den ubergelagerten hydrostatischen Druck zu messen und zu kompensieren was illustriert wird

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ULTRASTRUCTURAL OBSERVATIONS ON THE PAROTITIS AUTOIMMUNICA IN THE NZB/NZW HYBRID MICE

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(Received March 4 1977)

Abstract The lesions of the spontaneously occurring sialoadenitis in the parotid gland of New Zealand Black/New Zealand White (NZB/NZW) hybrid mice were studied with the light microscope and the electron microscope The inflammatory mononuclear cell infiltrates of the glands observed adjacent to glandular vessels and ducts were found to consist of several different cell types Lymphoid cells (large medium sized and small lymphocytes) were the most common but plasma cells histiocytes and macrophages were also encountered Furthermore lymphoid cells were frequently observed inside the basement membrane of acini as well as intercalated ducts Evidence of cell damage of the epithelium invaded by lymphoid elements included loss of cytoplasmic density vesiculation of the endoplasmic reticulum the appearance of cytoplasmic vacuoles and cellular lysis All the animals studied displayed moderate to advanced glomerular wire loop lesions in the kidneys The light and electron microscopic findings indicate that the parotitis in NZB/NZW mice is basically a vasculitis Duct cell proliferation and epimyo-epithelial island formation which are common features in Sjogren s syndrome are totally lacking in the material studied

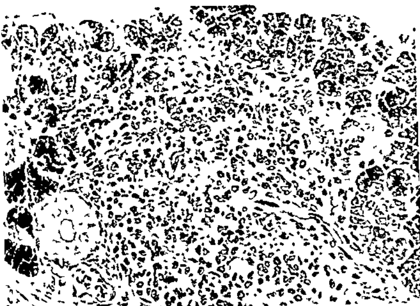
The autoimmune sialoadenitis (Mikulicz s disease benign lymphoepithelial lesion) in Sjogren s syndrome constitutes part of a systemic disease of considerable rheumatological and immunological interest (cf Shearn 1971 Mason & Chisholm 1975) The basic pathological changes occurring in the salivary glands of Sjogren s syndrome are however poorly understood (Boquist et al 1970 Kitamura et al 1970 Donath & Seifert 1972 Pirsig & Donath 1972) This is partly explained by difficulties in making early diagnosis and thus obtaining biopsies from the salivary glands at early stages of the disease The report of a murine type of Sjogren syndrome (Kessler 1968 Kessler et al 1971) is therefore of great interest and importance However, to the best of our knowledge there are no ultrastructural reports on cellular features in the salivary glands of this laboratory animal model of Sjogren s syndrome

The present report is an electron microscopic study on the parotid gland in the New Zealand Black/New Zealand White (NZB/NZW) hybrid mice with moderate and advanced kidney involvement (Mellors 1966 Talal & Steinberg 1974)

MATERIALS AND METHODS

NZB/NZW F1 hybrid mice obtained from the Medical Research Council Laboratories Animal Centre, Carshalton Surrey England were employed for the investigation Twelve animals (6 females age range 6–8 months 6 males age range 8–10 months) showing signs of terminal stages of their renal disease were fasted overnight and sacrificed The parotid glands and the kidneys were imm

This work has been supported by grants from the Swedish Medical Research Council (Project No B77 19X-0...) and the Mångberg Fund Umeå



1 One micron thick Epon section of the parotid d stained with toluidine blue A large mononuclear infiltrate is observed in the central portion of the section The surrounding acinar and striated duct cells appear comparatively unaffected Light micrograph ×560

2 Electron micrograph of an inflammatory cell in rate of the parotid gland Four small lymphocytes (*S*) as well as medium sized (*M*) and a large lymphocyte (*L*) are depicted in the section ×5300

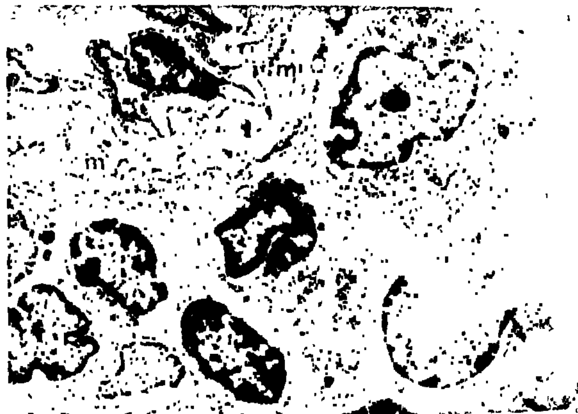


Fig 3 Electron micrograph of lymphoid cells surrounding a small arteriolus The cells are seen in the adventitia of the vessel in close proximity to the muscular media (*m*) ×5 100

diately removed from the animals and processed for the subsequent microscopical studies All animals used in this investigation showed glomerular wire-loop lesions of varying degree. The light microscopical and ultrastructural features of their autoimmune nephropathy have previously been reported (Emdin & Östberg, 1972)

Light microscopy

Specimens from the parotid glands were fixed in 10% formalin and embedded in paraffin wax Deparaffinized sections were stained with hematoxylin-eosin and the periodic acid-Schiff (PAS) reaction

Electron microscopy

Small pieces of parotid gland and kidney tissue were immediately fixed in 2 5% glutaraldehyde in 0 1 M phosphate buffer pH 7 4, for 2 hours at 0–4°C, rinsed in the same buffer, post-fixed in 1% osmium tetroxide, and embedded in Epon 812 Sections were cut on an LKB Ultrotome. Semithin (~1 μm) sections were stained with toluidine blue and used for the light microscopic identification of suitable areas for the thin sections Ultrathin sections were mounted on uncoated copper grids, and double contrasted with uranyl acetate and lead citrate; they were examined in a Philips EM 300 electron microscope

RESULTS

Light microscopy

The overall lobular architecture of the parotid gland was preserved, and the acinar cells were filled with distinct secretory granules In some focal areas, however, the gland parenchyma was replaced by lymphoid tissue (Fig 1) The size of the lesions varied from rather conglomerates of lymphoid cells to large areas



f Electron m crograph of a small arteriolus travers a mononuclear cell aggregate The external basement membrane of this vessel s pronounced and th ckened (*arrows*) ×6700

h diffuse lymphocytic infiltration The nonuclear cell aggregates often formed nductal and periarteriolar nodules Small ands of degenerated epithelial cells display g a dark agranular cytoplasm were oc sionally found within the lymphocytic ag gates

ectron microscopy

t the ultrastructural level the mononuclear ll aggregates were found to be composed of veral d fferent cell types Small and medium zed lymphocytes were the most conspicuous ells present (Fig 2) Based on purely mor hological grounds i e their size the lym ho c cells were categorized as small lympho- ytes (5–6 μm) medium sized (6–10 μm) and arge lymphocytes (10–12 μm) The spherical uclei of the small lymphocytes displayed a rom nent chromatin pattern nucleoli how ver were not often seen In the cytoplasm free ribosomes were abundant they were generally dispersed in the fairly electron dense hyaloplasm In the medium sized lymphocytes the nuclei were often irregular in shape Their chromatin was less condensed and prominent nucleoli were present (Figs 2 3) In the cyto- plasm mitochondria were sparse and only short channels of rough surfaced endoplasmic reticulum could be observed Polyribosomal clusters were frequently encountered The large lymphocytes finally displayed a rather electron lucid cytoplasm with a multitude of free ribosomes and sparse serpentine profiles of rough surfaced endoplasmic reticulum The nuclear heterochromatin was often concen trated to a rather thin peripheral rim (Figs 2 3) Frequently the lymphoid cells were dif ficult to classify and intermediate types were often observed Mature plasma cells with well developed Golgi regions and stacks of parallel dilated rough surfaced endoplasmic reticulum



Fig 5 Lymphoid cells of medium size (*L*) invading the epithelium of a secretory acinus Electron micrograph ×5300

were also encountered In some areas they were even abundant Non-phagocytic connective tissue cells as well as macrophages were also observed within the lymphoid aggregates Phagosomes containing degenerating cell structures were seen in many of the macrophages Moreover within these areas cell debris and necrotic cells abounded

Encircling many of the small vessels aggregates of lymphocytes and plasma cells appeared (Fig 3) The cells of these perivascular adventitial nodules were mainly concentrated in close proximity to the muscular media of the arterioli The external basement membrane of these vessels was frequently extremely well developed and fairly broad (Fig 4)

Lymphoid cells were often interspersed between the epithelial cells of the acini (Figs 5 6, 7) and also seemed to migrate between the intercalated duct cells (Figs 8 9) Many of the secretory cells, in close proximity to invading lymphoid cells, appeared damaged They played degenerative features such as creased cytoplasmic density, dilated or culated endoplasmic reticulum, cytopla vacuolization, and cellular lysis (Figs 5 6 The agranular dark epithelial cells within lymphoid aggregates observed in the microscope, could at the ultrastructural be identified as intercalated duct cells 10)

DISCUSSION

The pathological features in the major sal glands from patients suffering from Sjög syndrome are characteristic The feature clude marked lymphocytic infiltration d hyperplasia and the presence of so called epithelial islands (Morgan & Castleman 1 The cells of the islands consist of epit cells, derived from the ducts as well a



g 6 Electron micrograph of a secretory acinus Two mphoid cells are observed insinuated between the epithelial cells The intercellular spaces are markedly widened One acinar cell displays dilated cisternae of the ugh endoplasmic reticulum and a large vacuole is observed in another acinar cell ×5 100

lammatory cells (Boquist et al , 1970) In the ater stages of the disease there is a destruction of the duct epithelium, proliferation of intercalated duct myo-epithelial cells and infiltration, mainly by lymphocytes (Donath & Seifert, 1972) In the final stages, myo-epithelial cells produce a "basement membrane-like material causing extensive hyalin transformation of the islands The hyalin deposits, supposed to represent an antigen–antibody reaction, diminish after immuno-suppressive therapy (Pirsig & Donath, 1972)

In the NZB/NZW hybrid mice an autoimmune disease occurs spontaneously (Mellors, 1966, Greenspan et al , 1974*a*) The animals develop glomerular lesions analogous to those observed in systemic lupus erythematosus (SLE) (Emdin & Ostberg, 1972) Furthermore, these mice exhibit histo-pathological changes in both salivary and lacrimal glands similar to those observed in the glands from patients with Sjogren's syndrome (Kessler, 1968)

The mononuclear infiltrates in the parotid gland of the NZB/NZW mice (Kessler, 1968) observed in the present investigation were found to consist of a rather heterogeneous cell population However, lymphocytes of varying size constituted a major portion of the invading cells, but histiocytes, macrophages and plasma cells were also frequently encountered Lymphocytes were also detected inside the acinar basement lamina and thus in close contact with the acinar cells The latter often exhibited dilated cisternae of the RER, occasionally an extremely electron-lucid cytoplasm and/or pyknotic nuclei In addition, infiltrating lymphocytes were also observed in-





Fig 8 A lymphocyte invading an intercalated duct The lymphoid cell (*L*) is observed inside the basement membrane of the duct (*arrows*) Electron micrograph ×10 000

side the basement membrane of the intercalated ducts It therefore seems reasonable to assume that both the secretory cells and those of the intercalated ducts represent target cells for the infiltrating destructive lymphocytes

The small cell islands of epithelial cells observed within the lymphoid aggregates could, at the ultrastructural level, be recognized as intercalated duct cells However, no signs of intraductal cell proliferation and/or formation of myo epithelial cell islands were observed in the parotid glands of NZB/NZW mice, nor were any hyalin deposits present in the gland tissue Thus, from a histopathological point of view the autoimmune parotitis in NZB/NZW mice is very similar to the human sialoadenitis of the labial salivary glands where myoepithelial cell proliferation also is lacking (Shearn, 1971)

By employing immunofluorescence microscopic techniques, Greenspan and co-worker (1974*b*) have demonstrated that both thymus-derived lymphocytes (T cells) as well as bo marrow lymphocytes (B cells) are present the mononuclear cell infiltrates in the saliva glands of the NZB/NZW mice In the ear gland lesions these authors recorded that ly phocytes of the B cell lineage and plasma ce were the most conspicuous, whereas a grea proportion of T-cells was found in later sta when the infiltrates were enlarged in si Also in the salivary gland lymphocytic in trates in patients with Sjogren's syndro mixed T- and B cell aggregates have b recorded (Chused et al, 1974, Talal et 1974) According to Greenspan et al (197 the B cells and plasma cells could be invol with antigen recognition and/or early tis damage The T-cells on the other hand m



Fig 9 Marked lymphoid infiltration of an interc duct The lymphoid cells have penetrated the base membrane and the damaged epithelial cells d numerous cytoplasmic vacuoles Electron microg ×5 100



g 10 Portion of a small island of epithelial cells present ithin a mononuclear cell infiltrate The lymphoid cells re in close contact with the epithelial cells The latter splay a fairly electron-dense cytoplasm small Golgi complexes (*b*) and a sparse endoplasmic reticulum Desmosomal attachments are observed along the epithelial cell borders (*arrows*) Note the presence of two duct lumena (*D*) Electron micrograph ×8 300

ecome subsequently involved through sen-itization as a result of autoantigens release by B-cell cytotoxicity or as non-specific recruits n this delayed inflammatory reaction By ap-plying immunofluorescence microscopic tech-niques, it is possible to distinguish between T and B lymphocytes However, at the ultra-structural level the most common cell in the thymic gland, the thymic lymphocyte or T-cell, is indistinguishable in structure from small lymphocytes elsewhere Also the lym-phocytes of medium and large size within the thymus are structurally similar to medium and large lymphocytes in other lymphoid organs (Rhodin, 1974) Due to the difficulties in de-tecting differences in lymphocyte morphology by transmission electron microscopy, reports that B and T lymphocytes, examined by scan-ning electron microscopy (SEM), could be identified by their distinctive surface mor-phology, have been met with interest How-ever, Alexander et al have recently (1976) reported that it is not possible to reliably identify classes of lymphocytes *in vitro* ex-clusively on the basis of their SEM surface topography Thus, further refined electron microscopic immunocytochemical studies are required to reveal the difference between sur-face immunoglobulinpositive and -negative cells within the different sites in the salivary glands of the NZB/NZW mice

The histopathological changes occurring in the parotid glands of the mouse strain studied should be referred to as a vasculitis Al-though differing in features from the parotitis affection in the human Sjogren's syndrome, the similarities between the major salivary gland lesions in the NZB/NZW mice and those

of the labial salivary glands in patients with Sjogren's syndrome (Greenspan et al., 1974c) suggest common factors in pathogenesis and perhaps even in etiology.

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The skilful technical assistance of Miss *Siw Domeij* is gratefully acknowledged

ZUSAMMENFASSUNG

Die Läsionen von spontan auftretender Sialadenitis in der Ohrspeicheldrüse von mischerbigen Neuseeland Schwarz/Neuseeland Weissen (NZB/NZW) Mausen wurden licht- und elektronenmikroskopisch studiert Es stellte sich heraus, dass die entzündlichen einkernigen Zellen Infiltrate der Drüsen, die man in der Nähe von glandularen Gefässen und Gängen beobachtet ...

Ausserdem wurden lymphoide Zellen oft innerhalb der Basalmembran von Acini und auch innerhalb der da zwischenliegenden Gänge beobachtet Der nachweis der Zellenzerstorung des von lymphoiden Elementen durchsetzten Epithels umfasst den Verlust von protoplasmischer Dichte, Blasenbildung des endoplasmischen Reticulums, das Vorkommen von cytoplasmischen Vacuolen und zellige Lyse Alle untersuchten Tiere wiesen massige bis hohe glomerulare, drahtschleifige Lasionen in den Nieren auf

Die licht und elektronmikroskopischen Befunde indizieren, dass die Parotitis bei NZB/NZW Mausen in erster Linie eine Vasculitis ist Gang Zellen Wucherung und epimyoepitheliale Inselformation gewohnliche Zuge beim Sjögren Syndrom sind im untersuchten Material überhaupt nicht vorhanden

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ORGAN CULTURE STUDIES ON HUMAN SKIN AND CHOLESTEATOMA EPITHELIUM

Contact with Connective Tissue and Exposure to Vitamin A

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ract In tissue culture trunk skin from 8-day old chick showed a consistent change from keratinizing a more columnar non keratinizing epithelium under the ence of vitamin A acid using concentrations from 10 0 IU/ml Human adult postauricular and ear canal cholesteatoma membrane and human embryonic retained their keratinizing properties when exposed vitamin A acid at concentrations up to 150 IU/ml when the changes in the epithelium became obvious Treat ent of cholesteatoma ears with vitamin A ear drops is , to effect any change in the keratinizing properties the epithelium

present, little is known about the anisms controlling the development of a in type of epithelium and of the stimuli triggering its metaplastic changes Fell & Mel , (1953) noted that embryonic chick skin can be transformed from a keratinizing to a mucus secreting epithelium by exposure to excess vitamin A Weiss & James (1955) found that epidermal cell suspensions exposed to vitamin A only briefly (15 minutes) grow according to the metaplastic pattern even in normal unenriched media They postulated that this brief contact with vitamin A was sufficient to switch the cell differentiation into an alternative pathway

In experiments on embryonic rat skin from the tail and pads and from the trunk, New (1965) found 2–3 mm² large specimens to undergo certain changes under the effect of vitamin A Whereas control specimens developed thick stratified epidermis with abundant keratin vitamin A treated skin either failed to develop keratin, or if formed it was soon shed together with epidermal cells However, no mucous metaplasia occurred while some goblet cells were seen Barnett & Szabo (1973) reported inhibition of keratin formation with development of microvilli in the skin taken from the dorsum of a guinea pig s ear

Another mode of induction into different types of epithelium was noted in 5-day-old chick embryo tissue cultures by McLoughlin (1961) Various connective tissues specifically affected the differentiation and growth of epidermis On limb mesenchyme epidermis formed as normal, keratinizing epithelium, on gizzard mesenchyme it changed into mucus secreting instead of keratinizing epithelium, on heart myoblasts it grew as a thin 1 cell layer of endothelium type epithelium where as on heart fibroblasts it keratinized more heavily than in the controls

Experimentally, vitamin A has also been shown to play an important role in wound healing It stimulates the migration of epidermal cells and if cortisone has been used to impair wound healing decreasing the strength of collagen addition of vitamin A normalizes the system immediately (Ehrlich 1976) Vitamin A is thought to be of importance in glycoprotein synthesis and also to function as a carrier for sugar-phosphate compounds across the cell membranes

The use of vitamin A has also recently been suggested for the treatment of chronic middle

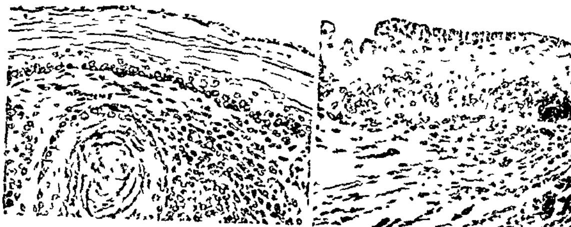


Fig 1 The effect of vitamin A acid on 8-day-old chick embryo trunk skin in 10-day old tissue culture The control specimen (*left*) shows strongly keratinizing squamous epithelium while the specimen exposed to vitamin A acid in a concentration of 20 IU/ml has lost all keratinizat and the epithelium has become more columnar in ty ×300

ear disease It has been reported to decrease infection and smell and to push back the cholesteatoma membrane (Duncan, 1976) Rarely, in individual cases, one of us (T P) observed disappearance of cholesteatoma epithelial remnants from the oval window when total removal had been judged to be too risky and the epithelial remains had been covered with crushed temporalis muscle tissue piece to await second stage surgery

In this work we have studied the growth capabilities of human postauricular skin, ear canal skin and cholesteatoma epithelium as well as various embryonic tissues in tissue culture and subjected the specimens to contact with various types of connective tissue as well as to different concentrations of vitamin A acid

MATERIAL AND METHODS

The following types of skin cultures were studied

1 trunk skin from 8-day-old chick embryos

2 postauricular and canal skin and cholesteatoma membrane from a human adult

3 postauricular and canal skin and cholesteatoma membrane from a human adult cultured on a piece temporal muscle or fascia

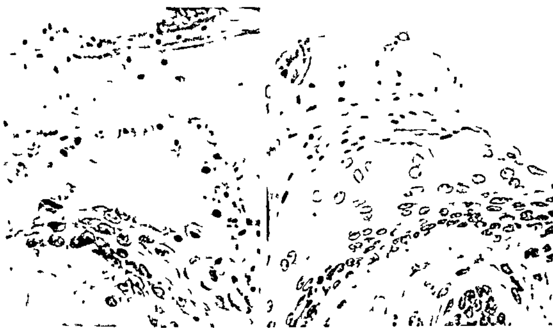
4 abdominal skin from a 190 mm CR human embryo

Postauricular and canal skin as well as pieces temporal muscle and muscle fascia were removed durin ear surgery and placed directly in culture medium Huma embryonic skin was obtained from a case of operati abortion

The skin was cut in approx 2×2 mm pieces in steri phosphate buffered saline under a dissecting microscope A layer of superficial dermis was left under the epidermis A Trowell type organ culture was used (Saxén 1966) Here the explants are cultivated on a metal grid at the medium–gas interface in a humidified incubator in an atmosphere of 5% CO_2 in air at 37°C Small pieces of Millipore filter (0 8 μm pore size 25 μm thickness) were placed on the metal grid and the skin explants were carefully placed on the filters epidermis upwards In experi- ... face ... the

Altogether 12 explants of chick skin 56 explants of adult human skin and 20 explants of embryonic human skin were cultivated

In cultures of chick skin BGJb medium (Biggers et al 1961 Orion Pharmaceuticals Finland) supplemented with 10% or 20% horse serum (Flow Laboratories) or with 20% horse serum +10% chick embryo extract was used These media were also used for cultivating human adult skin but equally good growth was obtained using a simpler medium Minimum Essential Medium (MEM) (Orion Pharmaceuticals Finland) +10% fetal calf serum (Microbiological Associates Inc , Bethesda Md) the lat



g 2 The effect of vitamin A acid on human adult ular skin on muscle after 15 days of tissue The control specimen (*left*) as well as the treated specimen (40 IU/ml vitamin A acid *right*) both show keratotic squamous epithelium with clear parakeratotic changes ×300

um was subsequently used in all experiments with adult as well as embr

young skin Vitamin A acid was first dissolved in absolute ethanol in a concentration of 3 mg/ml From this solution appropriate amounts were dissolved in serum by simultaneous vigorous shaking whereafter the serum was added to the medium

The medium was changed every other day in all experiments Chick skin was cultivated for 8 days and human skin for 8 15 and 30 days The explants were fixed in Zenker's solution and paraffin embedded The 6 μm serial sections were stained with hematoxylin and eosin with PAS or Alcian Blue

RESULTS

The experiments showed that all human epidermal tissues studied grew well in the MEM culture medium supplemented with fetal calf serum (FCS) showing good keratinization and forming rounded epidermal pearls Addition of fascia or muscle as a connective tissue matrix did not alter these properties Epidermis lined both the fascia and the muscle and in the specimens retained for 3 weeks it had a tendency to grow around the connective tissue pieces

Exposure of chick embryo skin to vitamin A acid resulted in a consistent change of the stratified squamous keratinizing epithelium into a more columnar type epithelium with no sign of keratinization (Fig 1)

Addition of 20% horse serum or 10% embryo extract, separately or together, to the BGJb culture medium did not affect loss of keratinization ability On the other hand, the change from keratinizing into columnar type epithelium was most marked when 10% embryo extract was used together with 20 IU/ml vitamin A acid

Staining with PAS gave a faint reddish tinge to the intercellular spaces but no proper secretory cells were seen All Alcian Blue stainings were negative in regard to acid mucopolysaccharides

Similar experiments with human epidermis gave negative results The keratinizing properties of postauricular and canal skin growing by themselves or on muscle bed with added



Fig 3 The effect of vitamin A acid on human adult ear canal skin on muscle in 12-day-old tissue culture The control specimen *(left)* shows marked keratinization with slight parakeratosis The specimen treated with vitamin A acid at 100 IU/ml *(middle)* shows a keratin pearl some damage to the nuclei in the epithelium The s men treated with vitamin A acid at 150 IU/ml (r shows toxic disintegration of the whole epithelium

horse serum or embryo extract, or both, were not affected by addition of vitamin A acid in ferent concentrations (Figs 2 and 3) The xic effect of vitamin A acid, nevertheless, became noticeable at 100 IU/ml and even marked using a concentration of 150 IU First the epithelial cells in the superficial l were detached from each other, then

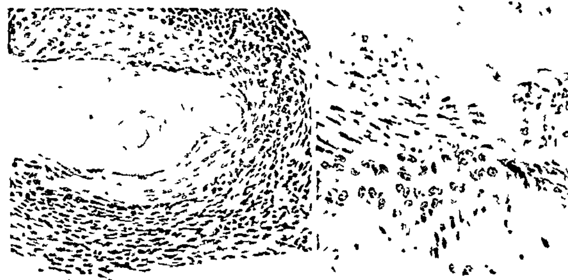


Fig 4 The effect of vitamin A acid on human 190 mm embryo abdominal skin in 15-day-old tissue culture The control specimen shows abundant keratinization *(left)* — A acid (150 xic effect

hole superficial cell layer was separated from e normal remaining basal cells and finally whole epithelium disintegrated

The effect of vitamin A acid was then tested skin of a human embryo, epidermis and rmis being taken from the abdominal area grown in MEM +10% FCS Keratiniza was seen to continue unabated at all con ions and toxic effect of vitamin A acid ld be observed using a concentration of 150 il (Fig 4)

DISCUSSION

The effect of vitamin A acid on chick embryo was clear and consistent when the initial nents were repeated with increasing ations of this preparation However, in A acid did not alter the keratinizing of human adult nor even of human embryonic epidermal tissues, the highest con centration used, 150 IU/ml, already exerting a toxic effect upon the epithelium

The idea of carrying out experiments with the two types of connective tissues, fascia and muscle as a potentially influential matrix for epidermal tissue transformation originally de rived from the experiments by McLoughlin, who showed that heart fibroblasts enhanced keratinization and myoblasts changed the epi thelium into a thin endothelium type lining It is precisely with fibroblast rich fascia that squamous epithelium always comes into con tact in tympanoplasties, while muscle comes into play only in obliteration procedures Clinical experience provides ample evidence that fascia is an ideal bed for resurfacing with keratinizing epidermis and this was fully borne out by the tissue culture experiments Un fortunately, however, neither crushed nor un crushed muscle affected the keratinizing epi thelium in any way It may be postulated that the tissue culture medium rapidly removes the possible enzymatic factors capable of epider mal transformation in crushed muscle whereas in vivo these factors might be retained long enough in a sufficiently high concentration to provide a booster for transformation of small remnants of squamous epithelium In this re spect, clinical experiments must be continued

At present, it seems obvious that in vitro fascia and muscle do not affect the properties of keratinizing epithelium, while vitamin A acid consistently transforms keratinizing em bryonic chick epidermis into a nonkera tinizing type This effect does not apply to hu man embryonic or mature keratinizing hu man epidermis and it is not likely that vitamin A ear drops at a non toxic concentration could exert any definite effect on cholesteatoma epithelium in chronic ears Some other factors govern the transformation of epithelium and their discovery would indeed greatly influence treatment of middle ear problems

ZUSAMMENFASSUNG

In Gewebskultur verursachte die A Vitaminsaure (Kon zentration 10–20 IU/ml) eine Transformation des keratini sierenden Plattenepithels zu nichtkeratinisierendem ko lumnarem Epithel bei Hautspezimens aus 8 Tage alten Huhnembryonen Die postaurikulare und Ohrenkanal haut Kolesteatomepithel und auch die embryonische Haut waren unverandert gleich unter Wirkung von A Vi taminsaure bis Konzentration zu 150 IU/ml wenn schon die toxischen Effekte der Saure sichtbar wurden Es ist unwahrscheinlich daß die A Vitaminsaure einen klini schen Effekt auf die Keratinisierung des Epithels verur sachen konnte

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THE PRE- AND POSTOPERATIVE ENG FINDINGS IN CLINICAL OTOSCLEROSIS AND THE LATE HEARING RESULTS

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act 125 patients suffering from otosclerosis under l oto-neurological investigations preoperatively and daily from the second to sixth day postoperatively abnormal ENG in the form of a spontaneous or posi nystagmus directional preponderance or a dimin caloric reaction could be found preoperatively in of the patients No statistically significant difference reen patients with or without abnormal ENG findings be shown in the late postoperative hearing results of 3 years in a series of different types of operations Nearly half of the patients had postoperative nystagmus On the second day nystagmus beat towards the operated ear in 22 3% of the patients On the sixth day there was nystagmus only in one third of the patients and it then beat towards the operated ear in only 12 1% of the pa tients and away from the operated ear in 21 6% No statistically significant correlation could be found between the nystagmus findings and the late hearing results after 3 years observation in this series of different ypes of operation

In clinical otosclerosis cochlear involvement often appear to a varying degree It is assumed that otosclerosis can be the cause of this damage to the cochlear part of the inner ear When otosclerosis damages the cochlea the question naturally arises whether otosclerosis can have a disturbing influence on the function of the vestibular organ too Fewer investigations have been published in this field than concerning the effect on hearing

Hulk & Jongkees (1950), Reinecken (1960), Fisch (1965) Meurman et al (1969) and Virolainen (1972) have shown different kinds of preoperative vestibular disturbances in 25–30% of patients suffering from otosclerosis Are these vestibular disturbances correlated with the late hearing results?

The modern surgical techniques for otosclerosis, with opening of the oval window covering it with a suitable material and then reconstructing the ossicular chain, always involve a certain risk to the labyrinth Opening of the labyrinth always brings about a reactive disturbance and a serous labyrinthitis, as is well known from the literature Subjective vertigo and spontaneous and positional nystagmus after operation are the commonest symptoms and signs of labyrinth irritation, and their severity can depend on the operative technique in question It is also possible that labyrinthine irritation after operation can influence the late hearing results

MATERIAL AND METHODS

The material consists of 125 patients who came to their first operation for otosclerosis between the ages of 18 and 64 (mean age 48 2) years Patients with other ear disease or head trauma and as subjects under medication were excluded Careful neuro-otological examination and hearing tests were made before operation Both spontaneous and positional as well as caloric induced nystagmus (modified Hallpike test) were recorded by an Elema Mingograph nystagmograph (a c condenser coupled time constant 5 sec direct ink writing) The spontaneous and positional nystagmus were recorded in the supine and right and left lateral positions before operation and again daily from the second to the sixth day after

Table I *The numbers of patients in the series*

Type of operation	Number of patients		Mean loss (dB) for 500 1000 2000 cps in operated ears: Air conduction	Air bone gap	4000 to 8000 mean (dB)
A Mobilization of the stapes	19 (15 2%)		47 0	27 9	51 6
		S D	12 1	7 9	21 9
B Anterior crurotomy and partial laminectomy	44 (35 2%)		48 1	29 5	49 3
		S D	12 5	9 3	20 5
C Stapedectomy and polyethylene prosthesis	46 (36 8%)		56 8	32 6	64 2
		S D	13 0	10 0	18 7
D Oval window fenestration and interposition	16 (12 8%)		51 0	30 9	57 8
		S D	11 3	7 4	20 0
Total	125 (100%)				

operation The nystagmus findings were considered positive if the speed of the slow phase exceeded seven degrees per second The hearing of the same patients was also examined 3 years after operation

The types of operation and the numbers of patients are shown in Table I Also the mean hearing loss before operation (air conduction and air–bone gap mean dB loss for 500, 1000 and 2000 cps and air conduction mean 4000 to 8000 cps) is shown In the mobilization series (A), there were 19 (15 2%), in the anterior y and partial laminectomy series (B) (35 2%), in the stapedectomy and polyethylene prosthesis series 46 (36 8%) and in the oval window fenestration and interposition series 16 (12 8%) patients The hearing loss of the contralateral ear was always less than in the ear to be operated There was no significant difference between each series concerning sex age or hearing loss

RESULTS

Six of 125 (4 8%) patients had preoperative subjective vertigo and 48 (38 4%) had tinnitus

There was a pathological ENG finding in the form of a spontaneous or positional nystagmus, directional preponderance or diminished caloric reaction in 39 (31 2%) of these patients Spontaneous or positional nystagmus was recorded in 13 (10 4%) patients Direc

Table II *The mean hearing results three years postoperatively in a series of different types of operation*

Type of operation		Air conduction mean (dB)	Air–bone gap (mean loss for 500 1000 2000 cps)	4000 to 8000 mean (dB)
A Mobilization of the stapes		38 7	20 3	43 5
	S D	12 7	12 6	9 5
B Anterior crurotomy and partial laminectomy		24 7	11 0	39 0
	S D	11 1	10 0	11 2
C Stapedectomy and polyethylene prosthesis		34 5	13 7	60 1
	S D	17 8	15 2	19 1
D Oval window fenestration and interposition		25 2	11 2	48 2
	S D	10 7	10 0	17 3

III *The numbers of patients in a series of different types of operation and the mean ng loss three years postoperatively in patients with abnormal and normal ENG before ion*

		Air conduction mean (dB)		Air–bone gap mean loss for 500 1 000 2 000 cps		4 000 to 8 000 mean (dB)	
of operation		Abnormal ENG	Normal ENG	Abnormal ENG	Normal ENG	Abnormal ENG	Normal ENG
Mobilization of the stapes		55 0	36 7	14 2	21 1	60 1	41 6
	S D	24 7	11 9	18 8	12 1	26 5	6 9
Anterior crurotomy and partial laminectomy		24 6	24 6	10 3	11 4	36 2	40 8
	S D	10 5	10 8	10 2	10 3	11 6	10 9
Stapedectomy and polyethylene prosthesis		33 6	35 5	16 0	12 7	57 5	61 1
	S D	20 2	16 2	16 4	15 2	19 7	19 2
Oval window fenestration and interposition		26 7	25 6	15 2	7 9	42 9	54 1
	S D	7 5	14 0	8 0	12 4	8 9	23 6

onal preponderance was shown by the canic tests in 14 (11 2%) and diminished caloric on in 19 (15 2%) patients

Patients who had vertigo in their case history before operation did not show more abnormalities in the ENG than those lacking this ymptom Pathological ENG findings were ound to be of a significantly higher degree in he patients complaining of subjective tinnitus han in the other otosclerotic patients There no significant preoperative difference in loss between patients with or without alities in ENG Hearing results after 3 ears of operation are shown in Table II are no significant differences in mean ir conduction (500 1 000, 2 000 cps) in the different series of operations The air–bone gap is significantly wider in series A (mobilization of the stapes) than in other series

The mean hearing results (air conduction, mean hearing loss in dB 500, 1 000 and 2 000 cps) mean air–bone gap 500, 1 000, 2 000 cps and mean hearing loss in dB by 4 000 to 8 000 cps in the subseries with and without preoperative abnormalities in ENG are shown in Table III There are no statistically significant differences in the hearing results between patients with or without preoperative abnormalities in the ENG The occurrence and the direction of nystagmus in the whole material from the second to sixth postoperative day are shown in Table IV One can see that on the second day there were as many cases with nystagmus beat towards the operated ear as away from it But subsequently the nystagmus became more infrequent and beat more and more away from the operated ear

The occurrence of postoperative nystagmus lasting more than 3 days after the operation in the series of different operations is shown in Table V There are in each series only about 15% patients who had postoperative nystagmus for 3 or more days When the hearing results 3 years after operation (Table II) were compared with these findings of postoperative

Table IV *The direction of nystagmus in the percentage of the whole material (125 subjects) from the second to the sixth day postoperatively*

The day after the operation	Nystagmus towards the operated ear (%)	Nystagmus away from the operated ear (%)	No nystagmus (%)
Second	22 3	22 3	55 4
Third	19 8	25 3	54 9
Fourth	16 8	31 5	51 7
Fifth	18 4	24 1	57 5
Sixth	12 2	21 6	66 2

Table V *The postoperative nystagmus*

Type of operation	No nystagmus or nystagmus during less than 3 days postoperatively	Nystagmus during 3 or more days postoperatively	Total
A Mobilization of stapes	16 (84 2%)	3 (15 8%)	19
B Anterior crurotomy and partial laminectomy	38 (86 4%)	6 (13 6%)	44
C Stapedectomy and polyethylene prosthesis	39 (84 8%)	7 (15 2%)	46
D Oval window fenestration and interposition	13 (81 3%)	3 (18 7%)	16
Total	106 (84 8%)	19 (15 2%)	125 (100%)

nystagmus there was no statistically significant difference between the hearing results and postoperative nystagmus in the whole material or in any special case of these operation series

DISCUSSION

On the basis of these results one can see that objectively verified vestibular disturbances very often appear in patients suffering from otosclerosis The changes were noticed in 31% of patients This is about the same rate as in earlier investigations (Hulk & Jongkees, 1950, Reinecken, 1960, Fisch, 1965, Meurman et al , 1969) Patients who had preoperative tinnitus had more abnormalities in their ENG recordings than others The reason for both signs could be an otosclerotic vascular change or a biochemical change of the inner ear fluids or some other reason not yet known However, the preoperative hearing or the hearing 3 years after operation was about the same in patients with or without tinnitus, which could also be a sign of damage to the inner ear One had also expected that in those patients who had abnormalities in their preoperative ENG, the hearing results would be worse than in those without any abnormalities Three years after operation one could not detect any differences between these series Earlier investigations have shown that there is a correlation between preoperative vestibular disturbances and high tone loss at 4000 and 8000 cps (Meurman et al , 1969, Virolainen 1972 Fisch, 1965) The results of this investigation are not in agreement with these findings On the second day after the operation in our cases, nystagmus was recorded in 44 6% of patients In half of the patients with nystagmus, beating was towards the operated ear and, in the other half away from it Nystagmus which beats towards the operated ear is a sign of a mild labyrinthine disturbance, whereas nystagmus which beats away from the operated ear is a sign of a greater disturbance was also the case in our investigation On the sixth day there was nystagmus only in 33 8% of patients, but in two thirds of them the nystagmus beat away from the operated ear This trend continued on each subsequent day Nystagmus which beats towards the operated ear disappears but nystagmus which beats away from the operated ear will continue This result differs from Ivstam's (1962) and Fi (1965) results, who found that the most quent type of nystagmus was directed wards the operated ear but their operative techniques were different Fortunately nystagmus findings in no way correlate with the final hearing results

ZUSAMMENFASSUNG

Es wurden 125 Patienten mit Otosklerose in der HNO Abteilung der Universität Turku Finnland pra und postoperativ untersucht Von diesen ze

Veranderungen in Elektronystagmographie in Form spontanem oder Lagenystagmus directional pre oder erniedrigter kalorischer Erregbarkeit die Halfte der Patienten hatten postoperativen stagmus Während des zweiten Tages schlug Nystag nach der Seite des operierten Ohres bei 22 3% der enten Wahrend des sechsten Tages gab es Nystagmus bei einem Drittel der Patienten und Nystagmus schlug h der Seite des operierten Ohres bei 1 2% der Pa und nach der Seite des unoperierten Ohres bei % der Patienten Es gab keine Korrelationen mit Findungen und spateren Gehorresultaten nach drei

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SKULL DISTORTION OF BONE CONDUCTED SIGNALS

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From the Departments of Audiology and Otolaryngology University Hospital Linkoping Sweden

(Received July 27 1977)

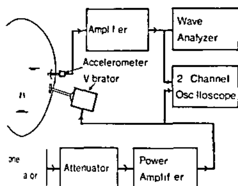
Abstract A previously essentially unknown type of distortion of bone conduction (BC) signal has been studied on the skulls of four human cadavers The method was based on a miniature accelerometer rigidly attached to the cranial bone converting the skull vibration close to the cochlea into an electrical signal which was analysed with regard to harmonic distortion The BC signals pure tones were presented by means of a high quality vibrator The distortion was found to be limited to the lower audiometric frequencies with a maximum around 500 Hz and of such a degree as to be able to significantly influence the results of BC audiometry The distortion is probably caused by nonlinear mechanical properties of the human skull

Pure tone audiometry by means of bone conducted (BC) signals is an established method in clinical audiology, which permits evaluation of the sensory neural function of the auditory system

However, BC audiometry has several limitations, some of which are determined by the equipment used, particularly the vibrator Normally, electromagnetic vibrators, primarily intended for use in BC hearing aids, are employed since they are easy to apply due to their small size Related to these small dimensions, however the frequency response of these vibrators is often poor and shows mechanical resonance in the range 200–600 Hz The harmonic distortion produced may influence hearing threshold measurements—at least at 250 and 500 Hz (Dirks & Kamm 1975) This nonlinear distortion of the vibrator increases with signal level, which thus contradicts the clinical requirement for higher available signal levels for BC audiometry

In an earlier investigation concerning BC stimulation for electrocochleography (Arlinger & Kylen, 1977), the ability of various types o vibrator to convert brief or transient electrica signals linearly to mechanical motion wa studied The vibrators were placed on variou locations on the skulls of human cadavers an the skull vibration patterns were recorded b means of a miniature accelerometer, rigidl attached to the promontory in the middle ear A pronounced nonlinear distortion was foun in the accelerometer output signal using ton bursts at 500 Hz but not at higher frequencie This distortion was present for all types c vibrators and all vibrator sites used It wa consequently suspected that this distortio was not due to the nonlinear behaviour of th vibrator but might be determined by nonlinea properties of the human skull

Kirikae (1959), when exploring vibratio patterns of human skulls excited by a vibrator, also noted that the acceleromet signal often contained components of hi frequencies superimposed on a signal of th same low frequency that was fed to the v rator He assumed that this phenomenon caused by harmonics of the low frequency ration at or close to the first resonance quency of the skull around 1 800 Hz K et al (1976) also came across the same distortion In a series of cancellation ex ments, i e psychoacoustic experiments w the level and phase of an air conducted pu tone was adjusted to cancel the auditory sation of a bone conducted pure tone found that for frequencies below 2 kHz the point of cancellation, i e when the mental component disappeared a



1 Block diagram of experimental equipment

remained The relative level of this was considerably higher than what could possibly be produced by the vibrator itself This distortion was amplitude depend ..." They propose two possible explanations ... near properties in the coupling between vibrator and skull, and the basic asymmetry of the cochlear response"

Thus the findings in these studies point to a type of nonlinear distortion which may occur ... stimulation in the low frequency region which is probably due to anatomical and physiological properties of the human skull Since this distortion was of such a magnitude that it could influence the results of BC audiometry, a more detailed investigation seemed imperative

METHOD

The intact skulls of four human cadavers were investi... During the experiments the cadaver was placed ... cot with the head supported by a wooden head rest covered by a cm thick layer of ...

A miniature accelerometer (Bruel & Kjær type 8303 ... 3.5 g) was used as a vibration pick up One end of a ... rod (length 35 mm diam 1 8 mm weight 1 g) was ... onto the accelerometer A small hole was drilled ... ntory in the exposed middle ear In this hole ... end of the rod was placed and firmly fixed to the ... tissue by means of cranial cement

The signal from the accelerometer was amplified (Bruel & Kjær 2603) and fed to a wave analyser (Radiometer ...3) for the harmonic analysis (see Fig 1) The wave analyser was used with its bandwidth set to its smallest ... z at −1 dB ±25 Hz at −50 dB)

Sinusoidal signals were generated by a tone generator (Hewlett Packard 241 A) attenuated (Hewlett Packard 350 D loaded by a 600 ohm resistor) and fed to a 15 W audio power amplifier (Vingtor K2 T) the output of which was connected to the vibrator (Bruel & Kjær 4810 Mini Shaker) This vibrator has a distortion of less than 1% in the frequency range 150–1 000 Hz at 10 g accelera tion This acceleration corresponds to a level of approxi mately 70 dB above normal threshold of hearing for bone conduction (Khanna et al 1976)

The input signal to the vibrator as well as the amplified accelerometer output signal were monitored on a 2 channel oscilloscope (Tektronix 561 A)

The Mini Shaker was fitted with a special adaptor made by a 60 mm long 6 mm diam aluminium rod with a plane circular tip surface having an area of approx 1 75 cm² A special holder was used for the vibrator by means of which the vibrator s position direction and static applica tion force could be adjusted A static force of approx 5 N was used in the experiments

The vibration levels used in the study were determined by means of calibration on a Bruel & Kjær 4930 Artificial mastoid with reference to the bone conduction threshold data presented by Flottorp (1972) Stimulation levels are thus given in dB HL

The harmonic distortion of the entire stimulation ap paratus was measured by placing the vibrator with its adaptor on the artificial mastoid the output of which was amplified (Brüel & Kjær 2603) and analysed with regard to harmonic components (Radiometer FRA3) The second harmonic at maximum signal levels used was −31 dB re the fundamental at 250 Hz/60 dB HL −35 dB at 500 Hz/65 dB HL and −35 dB at 750 Hz/70 dB HL At lower levels or at higher frequencies the second harmonic was 40 dB or more below the level of the fundamental All higher harmonics were always more than 45 dB below the fundamental

Since this type of skull distortion evidently only oc curred in the lower frequency range the experiments were limited to the frequencies 250 500 750 1 000 and 1 500 Hz Maximum stimulation levels of 60–70 dB HL were used comparable to what is found in commercially avail able audiometers The main experiments concerned the vibrator placed on the ipsilateral mastoid process but also forehead placement was studied

RESULTS

A common way of analysing nonlinear distor tion of a signal transmission link is to feed the link with a sinusoidal input signal of frequency f_0 Nonlinear transmission properties in the link causes harmonic distortion i e the output signal will no longer be a pure sinusoid but contain, in addition to the fundamental com ponent (at frequency f_0) also various har monics at frequencies $N \times f_0$ where N is an integer The degree of harmonic distortion is

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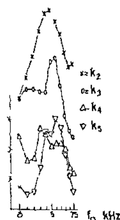
Abstract A previously essentially unknown type of distortion of bone conduction (BC) signal has been studied on the skulls of four human cadavers The method was based on a miniature accelerometer, rigidly attached to the cranial bone converting the skull vibration close to the cochlea into an electrical signal which was analysed with regard to harmonic distortion The BC signals pure tones were presented by means of a high quality vibrator The distortion was found to be limited to the lower audiometric frequencies with a maximum around 500 Hz and of such a degree as to be able to significantly influence the results of BC audiometry The distortion is probably caused by nonlinear mechanical properties of the human skull

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ε 3 Levels of the second–fifth harmonics as functions f frequency in the range 250–750 Hz Input voltage to brator corresponded to a BC signal level of approx 65 B HL at 500 Hz Mean values from three skulls

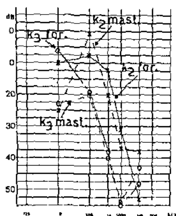


Fig 4 Levels of the second and third harmonics as functions of frequency with vibrator placed on the mastoid process and on the forehead BC signal level 60 dB HL Mean values of four skulls

50–750 Hz was studied in 50 Hz intervals on hree of the four skulls An input voltage to the ibrator corresponding to approx 65 dB HL at 00 Hz was used The mean levels of the nd–5th harmonics are shown in Fig 3 The ominating second and third harmonics both ave clear maxima at or very near 500 Hz

On all four skulls the vibrator was also placed on the forehead to examine the influence of vibrator placement Fig 4 shows the mean levels of the second and third harmonics for forehead and mastoid placements at 60 dB HL (data for 500 Hz obtained by linear interpolation from results at 55 and 65 dB HL) Only at 250 Hz a clear difference of any importance is seen At this frequency the harmonic distortion, measured in the accelerometer output signal, was considerably larger when the vibrator was placed on the forehead than when it was placed on the mastoid

Furthermore it was found that the vibrator was on average 20 dB less efficient in transferring the fundamental frequency of 250 Hz when placed on the forehead than when placed on the mastoid as seen in the accelerometer output signal level at this frequency At the higher frequencies this difference in efficiency between the two vibrator placements was smaller, the mean difference being 7 dB at 500 Hz, 4 dB at 750 Hz, 6 dB at 1 kHz and 0 dB at 1 5 kHz with the forehead consistently offering the poorest transmission to the skull

DISCUSSION

The results clearly confirm the previously noted existence of a serious distortion of bone conducted signals in the lower range of audiometric frequencies (Arlinger & Kylen, 1977)

The distortion of the electronic equipment, including the vibrator as used in the present study was significantly lower than the results obtained as presented in Figs 2–4 The fixation of the accelerometer to the brass rod and of the rod to the cortical bone was tested manually both before and after measurements and was always found to be very rigid In fact, a considerable force had normally to be applied to the rod to remove it after the completion of the measurements It may thus be concluded that no measurement artefacts due to the equipment and its use can explain the results The conclusion is further supported by the previously quoted findings of similar nature in other studies (Kirikae, 1959, Khanna et al, 1976)

The distortion obtained thus must be caused by nonlinear properties of the biological tis sues involved Khanna et al (1976) suggested that the soft tissues (skin and underlying tis sues) may not behave as a perfectly elastic coupling agent However, this suggestion is contradicted by the results of von Békesy (1960), who tested the linearity of vibration transmission through the skin at 400 Hz Using the web of the skin between the index finger and the thumb, placed between a vibrator and a vibration pick up attached to a large mass, he varied the vibration amplitude from 10^{-3} to 10^{-10} cm and found no evidence for nonlinear behaviour of the skin Further, in our previous study (Arlinger & Kylen, 1977) the vibrator was also placed directly on the exposed bone surface of the mastoid process after removing the soft tissues Although no quantitative measurements were performed a significant distortion of the same order of magnitude as that found in the present study was seen in the accelerometer output signal displayed on an oscilloscope

One possible explanation for the high harmonic levels obtained might be that pro nounced resonance phenomena in the skull give rise to a high amplification of weak harmonics present in the vibrator motion

Kirikae (1959) performed detailed mapping studies of the vibrational patterns of human skulls He found the first resonance frequency of the skulls to occur at 1 700–1 800 Hz One would then expect to find the dominant harmonic components in this frequency range This is not the case however As seen in Figs 2 and 3, at the signal frequencies of particular interest in this respect 250 and 500 Hz the distortion is dominated by the third harmonic (750 Hz) at 250 Hz and by the second harmonic (1 kHz) at 500 Hz i e by harmonics at clearly lower frequencies than the first re sonance of the skull

The origin of the BC signal distortion is con sequently most likely to be found in the bone tissue of the skull It is a well established fact that bone tissue has elastic as well as visco elastic and plastic properties (Evans 197 and thus may show a nonlinear relation be tween the driving vibrational force and vibra tion amplitude obtained

The distortion was found to be most pro nounced at 500 Hz The reason for this is not known but may be related to changes in the vibration pattern as the frequency is in creased Both Kirikae (1959) and von Békés (1969) found the skull to behave as a rigid body at very low frequencies (200–250 Hz) A 500–800 Hz one circular nodal line was found while at 1 000 Hz the skull vibrates with two nodal lines At higher frequencies the pattern becomes increasingly complex The fact tha the viscoelastic and plastic properties of tissue depend on the rate of mechanical ing i e on vibration frequency may also b an explanation for this finding

The distortion of BC signals thus seems originate from nonlinear mechanical erties of the human skull that can hardly eliminated The practical consequence of th findings is the risk of measurement error in B tone audiometry This risk is due to bility that a patient may respond to a while the fundamental component of stimulus tone is still below his threshold hearing thus giving rise to a false ai gap This risk is particularly evident for jects with sensorineural hearing loss of the lo frequency type, e g *Mb Meniere* but also flat losses

The existing IEC publication concerne with requirements on diagnostic (IEC 177), requires that each harmonic ponent should be at least 30 dB below the of the fundamental component for air tion In a study on detection of amplitude d tortion Gabrielsson et al (1976) concl that normal hearing subjects rarely 2 5% quadratic and 1 25% cubic distort i e when the second and third harmonics 32 and 38 dB below the level of the fu tal, respectively Based on these results maximum permissible harmonic level of dB re the level of the fundamental for BC

y seems a realistic minimum requirement

Applying this requirement to the present results makes it possible to define for each pure tone frequency a critical level, above which skull distortion reaches such a degree that it may cause measurement errors The critical levels thus obtained from the present results are the following 50 dB HL at 250 Hz, 25 dB HL at 500 Hz 50 dB HL at 750 Hz, 65 dB HL at 1 and 1 5 kHz

Hearing thresholds for bone conduction, at higher levels than these, must be evaluated with care Because of harmonic distortion in the bone vibrators commonly used in audiometry, BC measurement errors may occur at even lower signal levels, particularly at 250 Hz

CONCLUSIONS

1 A previously essentially unnoticed type of distortion of bone conduction signals has been investigated on the skulls of four human cadavers The distortion seems to be caused by nonlinear mechanical properties of the bone tissue of the skull

2 The distortion mainly concerns signals in the low frequency region, with a maximum around 500 Hz

3 The distortion reaches such a magnitude as to be able to cause significant measurement errors in bone conduction tone audiometry

4 Frequency dependent critical signal levels have been presented above which the distortion has been found to be significant Hearing thresholds for bone conduction obtained above these critical levels must be evaluated very carefully with regard to possible measurement error due to the skull distortion

ACKNOWLEDGEMENT

This investigation was financed in part through the Linkoping University Program Budget

ZUSAMMENFASSUNG

Eine fruher im wesentlichen unbekannte Verzerrung von Knochenleitungssignalen ist mit Hilfe von Vibrationsmessungen an intakten Leichenschadeln studiert worden Sinustone wurden uber einen guten Vibrationsgeber prasentiert Die Verzerrung war zum tieferen audiometrischen Frequenzbereich begrenzt mit einem Maximum bei 500 Hz und so groß daß sie die Resultate von Knochenleitungsaudiometrie beeinflussen mochte Die Verzerrung ist wahrscheinlich durch unlineare Eigenschaften des menschlichen Schadels verursacht

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HEARING LOSS RESULTING FROM PERILYMPH FISTULA

A Presentation of Two Cases

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(Received September 4 1977)

Abstract Two cases of perilymph fistula in the oval window are presented In such cases hearing loss may be severe and vertigo may or may not be present Early surgical intervention is recommended but one should not hesitate to explore a suspected ear, even if considerable time has elapsed

Many cases of suspected and verified perilymph fistula, spontaneous and traumatic, have been reported (Edmonds, 1973, Edmonds et al , 1973, Farmer & Thomas, 1976, Fee, 1968, Freeman & Edmonds, 1972, Freeman et al , 1974, Goodhill, 1971, Goodhill, 1972, Goodhill et al , 1973, Healy et al , 1974, Mulvaney & Montandon, 1974, Pullen, 1972, Stroud & Calcaterra, 1970)

The causal mechanism may be explosive or 'osive (Edmonds, 1973, Farmer & Thom- 1976, Goodhill, 1971, Goodhill et al , 1973) Explosive forces are initiated by all manoeuvres raising central venous pressure and cerebral spinal fluid pressure, which is then mediated to the perilymph, mainly through the ductus perilymphaticus An implosive force is raised air pressure reaching the middle ear through the Eustachian tube during the Valsalva manoeuvre

This paper will deal mainly with hearing loss resulting from perilymph fistula The audiograms were recorded according to the ISO Standard of 1964

MATERIALS AND METHODS

Case 1

A 38-year old woman had a right sided stapedectomy (piston technique) performed elsewhere because of otosclerosis Postoperatively she experienced vertigo and nausea which was worst on the fourth postoperative day After 2 more days her symptoms gradually subsided, but hearing tests showed the operated ear to be deaf The patient was told that the reason was a postoperative virus infection She was discharged from hospital after 16 days and was ill for another month She was still much troubled by whining tinnitus and with poor balance, especially early in the day, and she experienced vertigo every time she tried to clean her ear In addition she felt as though her right eye deviated involuntarily Since the hearing on the left side was also impaired, she obtained a hearing aid The vertigo subsided gradually but reappeared at once if she turned abruptly or blew her nose

Three months after the operation she was examined here as an out patient The audiograms were confusing and we could not be sure whether or not her right ear was stone deaf (Fig 1) Tuning fork tests were inconclusive, and the speech audiogram showed 0% score We feared that her ear was completely deaf and that her vertigo was caused by a perilymph fistula in the oval window Accordingly she was hospitalized one month later and a right sided exploratory tympanotomy was performed under local anaesthesia The oval window was found to be completely open without any trace of the stapes footplate The teflon piston was pointing towards the edge of the window and was replaced by a steel wire fat prosthesis At once the patient declared

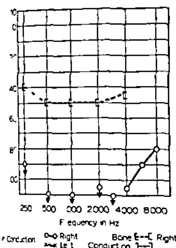


Fig 1 Initial audiogram from case 1 3 months after the stapedectomy O Air conduction masked 95–110 dB Bone conduction masked 105 dB

the noise in the room had altered in character Two days postoperatively a pure tone audiogram showed relatively good hearing in the ear and at speech audiometry she scored 80% at 100 dB SPL Four days later the hearing was even better, with a 40 dB mean loss in the speech frequencies, and a 100% score at 90 dB by speech audiometry (Fig 2) Her vertigo subsided and she left hospital 2 weeks after the operation

The hearing fluctuated, however, and since a conductive component in the loss seemed to remain she was readmitted about 2 months after the operation The ear was explored a second time but no explanation for the mixed fluctuating hearing loss was found The prosthesis was in place and everything seemed to be in order She left hospital on the fifth postoperative day and her hearing has continued to fluctuate The last audiogram showed a marked deterioration compared with our best recording

Case 2

A 25-year old fighter pilot skin dived to the bottom of a 4 m (13 ft) deep swimming pool During flying he had never had any difficulty in clearing his ears and during descent he never felt pain even though he did nothing active to equalize the pressure to his middle ears At the bottom of the pool he suddenly felt as if his right ear had become plugged He also experienced right-sided tinnitus but no vertigo The sensation of a plug in the ear and the tinnitus continued after the dive but he still experienced no vertigo in spite of flying a light aircraft afterwards Since the ear symptoms did not clear he was referred to one of us by the military physician 2 days later Except for slight injection of the nasal and pharyngeal mucosa the ENT examination disclosed nothing abnormal A pure tone audiogram, however showed a marked sensorineural hearing loss on the right side (Fig 3) Consequently he was put on the sick list and forbidden all physical effort The following week his hearing had not improved satisfactorily and he was therefore hospitalized on bed rest for another week During this time his hearing improved dramatically (Fig 4) but he still had a loss of 70 dB at 4 kHz which might have interfered seriously with his planned career as a pilot Therefore 2 weeks after the accident we explored his right middle ear and found a leakage of perilymph through the annular ligament in the inferior part of the oval window which was then covered with Spongostan The patient left hospital 8 days postoperatively and has since

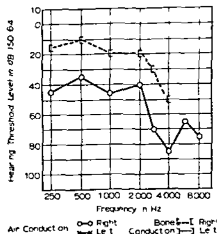


Fig 2 Best audiogram from case 1 6 days after closure of the fistula

had no trouble with his hearing The last audiogram, recorded 21 months after the accident, shows near normal hearing also at the 4 kHz frequency (Fig 4) The patient maintains that he had a 'notch" in the high frequency range of his audiogram on the right side before the accident, but we have not been able to obtain that audiogram for comparison

DISCUSSION

A definite preoperative diagnosis of perilymph fistula is difficult or impossible Most authors therefore recommend surgical exploration of the ear if a fistula is suspected (Edmonds, 1973, Edmonds et al, 1973, Farmer & Thomas, 1976, Fee, 1968, Freeman et al, 1974, Goodhill, 1971, Goodhill, 1972, Goodhill et al, 1973, Healy et al, 1974, McCormick et al, 1976, Mulvaney & Montandon 1974, Pullen 1972, Stroud & Calcaterra 1970) We fully agree with this procedure Perhaps one should allow a couple of days of complete bed rest in case spontaneous closure occurs If vertigo subsides and hearing is restored one may postpone surgical intervention The patient must be strongly advised to avoid the Valsalva manœuvre, vigorous nose blowing and, if possible sneezing pre and postoperatively It is equally important to avoid any other manoeuvre tending to raise the central venous and cerebrospinal fluid pressure such as heavy lifting or straining at stool Should any of these manoeuvres occur in the postoperative phase the fistula may re open and another operation may become necessary (Fee 1968 Pullen 1972)

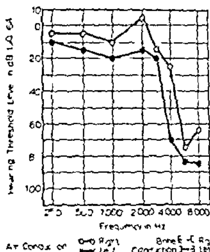


Fig 4 ● Immediate preoperative audiogram from case 2, 2 weeks after the accident ○ Most recent follow-up audiogram from case 2 21 months after the accident As hearing loss was purely sensorineural the bone conduction curves are omitted for clarity

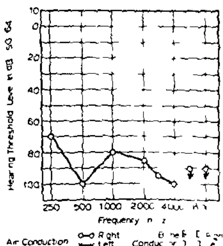


Fig 3 First audiogram from case 2 ? days after the accident As hearing loss was purely sensorineural the bone conduction curve is omitted for clarity

Our second patient was strongly discouraged from further diving but we found no reason to interfere with his flying career To experience in flight a sudden pressure change corresponding to a dive to a depth of 4 m (13 ft) of water would mean complete loss of cabin pressure at a 24 000 ft altitude a very unlikely event

Hearing loss and vertigo from perilymph fistula seems to be a growing problem among divers, as reflected in the literature Diving mammals seem to escape the problem through adapted anatomical peculiarities (McCormick et al, 1976)

There are two points about these cases worth stressing

1 Perilymph fistula may lead to profound hearing loss even greater than in cases with destruction of both the incus and the supra structures of stapes or with complete fixation of the stapes footplate In the first pa

ient we initially feared that the ear might be ompletely deaf

2 It seems as though leakage of perilymph nay exist for a long time without the patient leveloping infective labyrinthitis This view is upported by our observations during several tapedectomies where stapedolysis had been performed years earlier and where leakage of perilymph through the oval window was still present at the time of our operation None of those patients showed any sign of past or pres nt infective labyrinthitis nor did the history suggest it Nevertheless, we suggest not to hesitate too long before exploring an ear suspected of perilymph fistula, since much may be gained and little lost with this procedure

ZUSAMMENFASSUNG

Zwei Falle mit perilymphatischen Fistelbildungen am r

emp ehlen Eben in Fallen wo der Zustand lange ge dauert hat ist eine abwartende Haltung nicht zu empfehlen.

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SPEECH AND PURE TONE AUDIOMETRY AS A SCREEN FOR EXAGGERATED HEARING LOSS IN INDUSTRIAL CLAIMS

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(Received December 6, 1976)

Abstract. We examined 596 patients referred with possible noise induced hearing loss by conventional and cortical evoked response audiometry. Discrepancies between the two tests identified 121 (20.3%) as exaggerating their hearing loss. We then studied the validity of simple conventional tests which would be available in primary diagnostic facilities in screening for the exaggerators we had identified. By selecting those whose puretone average (for 0.5 and 1 kHz) was more than 10 dB different from the speech reception threshold or whose initial puretone threshold at 0.5 kHz was 40 dB or greater, we identified 112 (93%) of the exaggerators, at the cost of additional examination of 209 (35% of the total) false positive. However this screening means that 46% of the claimants could be evaluated completely in a primary diagnostic facility and only a small fraction of the exaggerators should be overlooked.

Hearing loss caused by exposure to industrial noise is one of the major public health issues concerning Otolaryngologists today. In the Province of Ontario, Canada, occupational hearing loss first became compensable through The Workmen's Compensation Board—essentially an industrial insurance Board—in 1948 and, after a slow start, claims have nearly doubled in each of the last three years. There were about 900 claims in 1974, 1 500 in 1975, and 2 500 are expected in 1976. The proportion actually receiving compensation has remained constant.

In Ontario there is an uneven distribution of population and noisy industry: 80% of claims currently come from more than 300 kilometers north of Toronto, although only 20% of the population live so far away. To compound matters, almost all of the elaborate testing facilities are in the southern towns.

Whenever compensation is directly re to severity of disease, great care must be t in the accurate quantification of the disorder hearing loss is no exception. It is generally accepted that the rate of exaggerated loss in industrial claims is between 15 and 20% and the degree of exaggeration is usually more than 15–20 dB, almost always on top of genuine coexisting hearing loss. Howe whilst these figures do not seem great w translated into compensation awards represent a considerable sum of money at stake. Previous work from our laboratory (Alberti & LeBlanc, 1976) has shown that average lifetime saving per claim per exaggerator is approximately $5 000. Of anticipated 2 500 claimants in 1976 about will be exaggerating, and if undetected v over their lifetime cost an additional $1 their detection could save almost $90 000 nually.

The Compensation Board have, up to now requested that most claimants travel to major centre in order that accurate evaluati can be undertaken. This is a significant disturbance and expensive dislocation of people the justification for which lies in the need detect exaggerators, compounded by the

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sence of sophisticated testing facilities in the northern part of the Province

The purpose of this communication is to describe investigations we have undertaken to attempt to identify potential exaggerators by means of simple hearing tests thus separating those who should be tested in sophisticated facilities from those whose hearing has been accurately quantified by simple tests This goal is a common one in many countries for few have adequate facilities for full tests of all workers it is necessary to determine who needs them

MATERIAL

In our laboratory we have seen about 1 500 claimants in the past 5 years and kept careful records of our findings which have been com puter stored for further study Our goal was to see if examination of puretone thresholds alone or comparison of puretone thresholds and speech reception thresholds would have provided an appropriate screening test for exaggerators

A group of patients was chosen for this study in whom we have accurate puretone thresholds to act as a base They were then compared with a group of exaggerators in whom we believe we were successful in es tabl shing an accurate final puretone thresh old Both groups had cortical electrical re sponse audiometry (ERA) undertaken in addi tion to other tests We have used ERA in neurologically normal adult military and indus trial hearing loss claims since early in 1969 (Alberti 1970 Alberti et al 1974) for a vari ety of reasons sometimes on the total popula tion sometimes sampling and sometimes only when suspicious Evoked response and rou tine audiometry has always been performed a d interpreted by a different tester If the re sults differed tests were repeated In all in stances the figures taken as the final true threshold was the result of at least two dif ferent tests agreeing i e conventional pure tone threshold and evoked response matched or evoked response and speech reception threshold matched

In an earlier unpublished study in non exaggerating industrial patients puretone threshold results and speech reception thresh old had been compared utilizing the recorded Los Angeles Foundation W1 and W2 tests—in an attempt to verify the Fletcher formula for our patients In the sloping audiogram typical of noise induced hearing loss there was a closer relationship between the SRT and puretone average hearing at 500 and 1 000 Hz than there was among the puretone averages at 500 1 000 and 2 000 Hz although the pure tone average at 500 and 1 000 Hz was 2½ dB (±7 dB) better than the speech reception threshold we feel that SRT can be used as a reliable estimator of lower frequency puretone thresholds

Patients who initially exaggerated their hearing loss were identified by means of ERA tester suspicion and discrepancies between the various hearing tests Exaggerated hearing loss was defined as a greater than 10 dB hear ing discrepancy between the summed average hearing loss of 500 and 1 000 Hz in both ears on the initial test results in our laboratory and the final ultimately accepted threshold This final threshold was usually arrived at after ERA s had shown the initial threshold to be 10 dB or more too high

RESULTS

The entire population who had both conven tional and evoked response tests undertaken consisted of 596 patients 121 of whom (or 20 3%) had exaggerated hearing loss

(*a*) *SRT puretone threshold comparisons*

Detailed frequency distribution studies were made of the conventional puretone threshold/ SRT relationships Preliminary inspection of the results suggested that a reasonable cutoff point was an agreement within 10 dB or less to

Table I *Puretone (PTT)/SRT discrepancy as a screen for exaggerated hearing loss*

PTT/SRT	Non exaggerators	Exaggerators	
≤10 dB	462	46	508 pass
≥11 dB	13	75	88 fail
Totals	475	121	596

indicate no exaggeration, or alternatively, a discrepancy of more than 10 dB between the speech reception threshold and the puretone average at 500 and 1000 Hz frequency indicated exaggeration This was put to the test The results are shown in Table I

It can be seen that of a total population of 596 patients 79 3% had puretone threshold and SRT responses of 10 dB or closer and of those 9 1% (46/508) were in fact exaggerators Of the population where the puretone threshold and speech reception threshold were greater than 10 dB apart, 14% (13/88) were non exaggerators and 85 2% (75/88) were correctly identified as exaggerating Overall, of those who exaggerated 62% (75/121) were correctly identified

However, whilst helpful, this still means that 38% of those who exaggerate are missed —a figure which we find unacceptably high

(*b*) *Puretone threshold at 0 5 kHz*

The puretone threshold at 500 Hz was examined as a screen for exaggerated hearing loss Frequency distributions suggested a 40 dB cutoff to attempt to separate patients into those who exaggerated and those who did not

Looking at this interaction independently (Table II) we found that 52% of the population had a puretone threshold of 40 dB or worse at 500 Hz and a third of these were exaggerators (103/310) However, this cutoff did identify 85% of all exaggerators—better than the SRT test, but still unacceptably low

(*c*) *Combined SRT/PTT and 0 5 kHz criteria*

The tests were combined in an attempt to improve the sensitivity Table III shows the 500 Hz test applied to the 508 patients who 'passed the SRT/PTT test, a group which included a residium of 46 so far unidentified exaggerators If the 500 Hz, 40 dB cutoff is applied to this group along (Table III) a further 37 patients with exaggerated hearing losses are identified, only 9 of the total of 121 (7 4%) are now missed at the price of a false identification of a further 196 patients

As we combine the tests by failing all those who pass the first but fail the second criterion we correctly identify 112 of a total of 121 exaggerated hearing losses or 93% of the total whilst falsely identifying 209 people (Table IV) Thus of 596 original patients 44 6% (266/596) are correctly identified as giving accurate thresholds, 53 8% (321/596) are identified as giving inaccurate thresholds, and under half of these (112/321) are acutally exaggerating Thus, by means of these two simple tests the number of patients who have to travel to a 'sophisticated centre may be almost halved at a considerable economic and social saving but the number of patients with missed exaggerated hearing loss is kept down to what r

Table II *40 dB threshold at 0 5 kHz as a screen for exaggerated hearing loss*

0 5 kHz threshold	Non exaggerators	Exaggerators	
≤39 dB	268	18	286 pass
≥40 dB	207	103	310 fail
Totals	475	121	596

Table III *Application of 40 dB 0 5 kHz threshold to those passing test in Table I*

0 5 kHz threshold	Non exaggerators	Exaggerators	
≥40 dB	266	9	275 pass
≤39 dB	196	37	233 fail
Totals	462	46	508

Table IV *Application of SRT/PTT and 0 5 kHz criteria to the entire study group*

	Non exaggerators	Exaggerators	
Pass both criteria	266	9	275
Fail either criterion	209	112	112
Totals	475	121	596

probably an acceptably low figure, i e 7 4% of the exaggerators or 1 5% of the total population

It has previously been shown by Klockhoff et al (1974) that a hearing threshold of 40 dB or worse may well indicate the presence of other ear disease Thus the false positive group identified within the narrow context of exaggerated hearing loss, may confirm patients with other ear disease, much of it not previously diagnosed These patients stand to benefit from further diagnosis at a secondary centre

To complete the story, the criteria outlined were applied to the total population of patients referred to us with noise induced hearing loss alone These are patients who did not necessarily have ERA but were sent by the Compensation Board for assessment The double test criteria were applied to the total computer bank of 1456 patients Approximately 46% passed both tests and thus potentially do not need sophisticated test facilities, and only 54% need be sent to a major centre—a vast improvement Further studies are in progress to see if screening in a primary centre will have the same value as it has in a fully equipped diagnostic centre such as ours

CONCLUSIONS

Careful screening by means of puretone and speech reception thresholds in NIHL may limit the need for testing in major centres always with the proviso that if there is a history of ear disease, a discrepancy between the hearing in the two ears, or tester suspicion the patient be referred further

ZUSAMMENFASSUNG

Wir untersuchten 596 Patienten die wegen der Möglichkeit eines larmbedingten Hörverlustes überwiesen worden waren durch konventionelle Audiometrie und kortikale hervorgerufene Potentiale Diskrepanzen zwischen beiden Tests identifizierten 121 (20 3%) Fälle die einen übertriebenen Hörverlust vorspiegelten Dann prüften wir die Validität einfacher konventioneller Testmethoden jener Art wie sie in primären diagnostischen Laboratorien zur Verfügung stehen indem wir nach den Übertreibern suchten die vorher identifiziert worden waren Wenn Fälle mit einer Differenz von mehr als 10 dB zwischen durchschnittlicher Reinton (für 0 5 und 1 kHz) und Sprachrezeptionsschwelle ausgewählt wurden oder jene deren initiale Reintonschwelle (bei 0 5 Hz) 40 dB oder mehr betrug konnten wir 112 (93%) der Übertreiber identifizieren allerdings auf Kosten von 209 (35% des Gesamtmaterials) falsch positiven Fällen Dennoch zeigt die Bedeutung dieser Testprozedur daß 46% der Antragsteller komplett in einem primären Diagnoselabor ausgewertet werden konnten und nur ein geringer Teil der Übertreiber übersehen wurde

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PERIPHERAL VASOCONSTRICTION IN THE RAT IN RESPONSE TO SOUND

II *Dependence on Rate of Change of Sound Level*

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(Received July 1 1977)

Abstract Vasoconstrictions elicited by sound were studied in the non anaesthetized rat Arterial pulsations in the tail were recorded by a non invasive technique On slightly heating the animal, the tail vessels became dilated An 80 dB SPL noise burst caused a decrease in pulse amplitude usually to less than 10% of the pre-stimulus value It was found that 4 s bursts of 80 dB SPL noise with rise times 1 10 or 100 ms were equally efficient in producing vasoconstriction If the rise time was longer, 1 s the vasoconstriction was significantly smaller It was pointed out that the feedback control of the stimulus noise provided by the acoustic middle ear reflex would contribute to enhancing rapid variations in sound level and thereby form part of a physiological explanation for the present findings

Over the years, an increasing interest has been focused on the relation between the auditory system and the autonomous nervous system Two incentives to this development may be mentioned Firstly the possibility of noise being a health hazard and secondly, the demonstration of the richness of sympathetic innervation of the inner ear (Spoendlin & Lichtensteiger, 1966, Densert 1974) The two basic questions relevant to these problems are largely unanswered (*a*) How does sound influence the activity of the autonomous system? and (*b*) What is the effect of the sympathetic nerves on the ear?

Research in this field has been hampered by the lack of suitable experimental preparations A recently described animal model has, however, been found suitable for an approach to the first of the questions mentioned above (Borg, 1977*a* *b*) It was shown that the blood flow in the tail of the non anaesthetized slightly heated rat was influenced by a sound stimulus to the ear in a reproducible fashion and systematically dependent on sound characteristics

The vasoconstriction following a sound burst was found to be proportional to sound level from hearing threshold over at least 80 dB (Borg, 1977*a*) and dependent on duration of the burst corresponding to a time constant for temporal integration in the order of 0 1 s (Borg, 1977*b*)

The aim of the present study is to continue the analysis of the role of acoustic features for peripheral vasoconstriction The sensitivity of vasoconstriction to rate of change of sound level has been investigated by using noise bursts with rise times in the range 1 ms to 1 s

METHODS

Two series of experiments were performed Ten adult, male rats participated in each One of the animals was experimentally naive and animals took part in both sessions The two sessions were identical except for the choice of rise time values

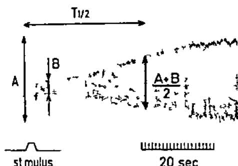


Fig 1 Recording of arterial pulsations obtained with a non-invasive technique from the tail of the non anaesthetized rat Decline in pulse amplitude is elicited by a 4 s burst of 80 dB SPL noise with 1 s rise and decay times The vasoconstriction is quantified by $T_{\frac{1}{2}}$ time from onset of sound until pulse amplitude is halfway normalized

Since a detailed presentation of the stimulus and recording system has been given earlier (Borg 1977*a*) the following description of these systems will be brief During the experimental session the animal rested on a regulated heating pad in an individually adjustable net tube The blood flow in the tail was assessed in terms of pulsations recorded by a rubber balloon connected to a volume-sensitive transducer (Elema 510 C) In room temperature the tail artery was constricted and pulsations were minimal A moderate heating was necessary to induce vasodilatation The acoustic–vascular reflex manifested itself as a decrease of pulse amplitude, usually to less than 10% of the prestimulus value The vasoconstriction was quantified as the duration of the response from the start of the sound until the amplitude of the pulsation had returned halfway to pre stimulus level ($T_{\frac{1}{2}}$ of Fig 1) The reasons for using the duration of the response rather than the change of pulse amplitude as a measure of the vasoconstriction have been presented earlier (Borg, 1977*a*) A Lansing L75 loudspeaker placed 10 cm in front of the rat was used to deliver the sound stimuli

The stimulus consisted of a burst of a broad band noise with maximum energy between 3 and 15 kHz (see Fig 2, Borg 1977*a*) Its sound level was 80 dB SPL (sound pressure level, re 20 μPa) The total duration of the burst was 4 0 s, its time for linear rise (and decay) being 10, 100, or 1 000 ms (in the first series of experiments) and 1, 10 or 100 ms (in the second series of experiments) During each session all of which lasted 4–12 h the differently shaped stimuli were presented several times (3–5) in random order with an interval of at least 15 min

RESULTS

A typical recording of a tail artery pulsation during acoustic stimulation is shown in Fig 1 The acoustic–vascular reflex response to an 80 dB SPL noise burst with a 1 s rise time (and decay time linear) is evident as a decline of pulse amplitude After a delay of approximately 1 s the pulse amplitude diminishes returning to the normal value after about 1 min The responses were quantified by $T_{\frac{1}{2}}$ which denotes the time lapse from start of stimulus until the pulse amplitude had returned halfway to pre-stimulus value

The dependence of the vasoconstriction on rise time in the first series of experiments is illustrated in Fig 2 The thin continuous lines

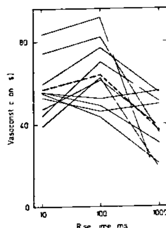


Fig 2 Duration of vasoconstriction as a function of rise time (10 100 and 1000 ms) of a 4 s burst of 80 dB SPL noise Each thin continuous line represents one animal in which up to 5 determinations at each rise time were obtained Heavy broken line shows mean values

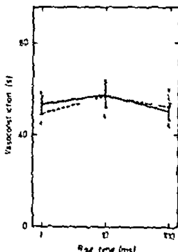


Fig 3 Duration of vasoconstriction as a function of rise time (1 10 and 100 ms) of a 4 s burst of 80 dB SPL Average and standard error of the mean — Median and semi interquartile range -

represent average responses to 4 s noise bursts with rise times of 10, 100, and 1 000 ms in each of the ten animals The heavy broken line shows mean values It is seen that bursts with rise times of 10 and 100 ms give about equal responses, whereas those having a 1 s rise time are usually followed by shorter vasoconstrictions The difference between 100 and 1 000 ms responses is statistically significant (Student t test at 1%-level) The difference between responses for rise times of 10 ms and 1 000 ms is significant at the 5%-level (Student t-test) One may also get the impression that bursts with 10 ms rise times are less efficient in producing vasoconstriction than are those with 100 ms rise time

In order to analyse the short rise time range (1–100 ms) further the second series of experiments was performed Fig 3 shows mean values and standard error together with median and semi interquartile ranges from experiments in 10 rats It appears that bursts with rise times from 1 to 100 ms are equally effective in eliciting vasoconstriction

In conclusion, sound bursts which are slowly changing in level elicit smaller vasoconstrictions than do bursts with rapidly changing intensity

DISCUSSION

The results show that a 4 s noise burst (80 SPL) elicits a smaller vasoconstriction wh. the rise- and decay times are long, 1 s thar when they are short, 1, 10, or 100 ms The bursts with faster rise are, on the other hand equally efficient This difference is not a con sequence of the shorter effective duration of the burst with 1 s rise and decay time (pla at 80 dB SPL, 2 s) It has been shown t. ly, that noise bursts with 1 s and 4 s d give almost identical vasoconstrictions as lon. as they have equal rise times (Borg 1977b)

Evidence exists indicating that rise time i important for the ability of sound to influenc sleep LeVere et al (1976) showed that sou with rapid onset (relay controlled) was effective in causing disturbance of sleep than slowly rising (7 5 s rise time) sound On t other hand, according to Nixon et al ... noises with very slow onset (15 s) appear more annoying than suddenly occurring This observation was explained by the a sumption that the increasing level is perceiv as being produced by an approaching objec An adequate comparison between anno change of sleep stage, and vasoconstrictic can be made only when observ... identical stimuli are available This is unf tunately not the case

The low sensitivity of the acoustic-va reflex to slowly rising stimuli may be cau by negative feedback control in the lower tory system A negative feedback effect on acoustic signal or on the neural message tend to enhance rapid variations in ampl compared with slow variations The follo remarks will be limited to the best known the many feedback systems in the aud pathway, the acoustic middle ear muscle flex The intensity and frequency of used in the present experiments are lare within the ranges controlled by the middle muscles in animals (Borg 1972) If the time is short, a significant part of the acous energy will escape the reflex attenuation p vided by the muscles due to their relat

low dynamic properties (Borg, 1976) When the middle ear muscles are excited by a tone with 2–10 ms rise time, full attenuation is provided after 200 ms in humans, and after ms in rabbits In rats, the figure is not known but most probably full attenuation is ded after 50 ms or somewhat less When onset of sound is slow, the time to full effect of the feedback control is somewhat longer No measurements with 100 ms and 1 s rise times are however, available Thus, the attenuation of the stimulus provided by the ear muscles may be suggested to con- to decrease the efficiency of bursts 1 s onset in comparison with bursts with shorter rise time A more complete account of the underlying mechanisms can not be pres ented until the neuronal pathway of this acous- tic vascular reflex is established

In summary, the present experiments show the acoustic–vascular reflex in the tail of has rate sensitive properties

ZUSAMMENFASSUNG

An nichtanasthetisierten Ratten wurden die durch Ton geräusche ausgelosten Gefäßkontraktionen untersucht und zwar durch Aufzeichnung der arteriellen Pulsationen im Schwanze mittels einer nichtinvasiven Methode Durch leichte Erwarmung des Tieres wurden die Blutgefäße im Schwanz erweitert Es konnte gezeigt werden daß Gerauschstoße von 80 dB SPL eine Abnahme der Pulsamplitude bewirken und zwar im allgemeinen auf weniger als 10% des Wertes vor der Stimulation Weiter wurde festgestellt daß Gerauschstoße von 4 s Dauer und 80 dB SPL bei Steigzeiten von sowohl 1 wie 10 oder 100 ms praktisch die gleichen Gefäßkontraktionen hervor rufen War jedoch die Steigzeit langer (1 s) so war die folgende Gefaßkontraktion signifikant geringer Eine physiologische Erklarung dieses Verhaltens konnte darin gesehen werden daß die Ruckkopplungskontrolle durch den akustischen Mittelohrreflex auf den Gerauschstimu lus dazu beitragt steile Anderungen im Gerauschpegel zu vergroßern

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REFLEX INHIBITION AUDIOMETRY

A New Objective Technique

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Abstract The reflexive eyeblink elicited by a tactile stimulus is inhibited if an auditory stimulus precedes the eliciting stimulus by 100 msec With adult subjects the threshold for this effect was found to be sufficiently low to suggest that reflex inhibition may be useful in objective audiometry A preliminary investigation with infants and children showed that the inhibitory process is present, though variable in children as young as 6 weeks old

One of the more difficult problems facing the audiologist is the evaluation of the nonverbal child Although several objective techniques are available, each has some limitation, such as cost in equipment or time, or unsuitability for very young or multiply handicapped patients We report here a new objective technique which shows promise of overcoming some of these barriers In this test one assesses hearing acuity by determining whether the reflexive eyeblink (elicited by tactile stimulation of the face or cornea) is inhibited by an acoustic stimulus which precedes the non acoustic reflex eliciting stimulus by a fraction of a second This technique, which we have named reflex inhibition audiometry, requires no active cooperation on the part of the patient nor does it involve learning or conditioning These two characteristics in particular would recommend its use with the difficult-to-test child

Reflex inhibition audiometry had its origin in a series of investigations of the acoustic startle reflex in the rat This reflex as measured with a stabilimeter was found to be inhibited if a single relatively weak pulse o noise preceded the more intense startle stim ulus by 20–500 msec (Hoffman & Searle 1965) Further investigation showed this in hibition to be robust, persisting after hours o presentation of the inhibitory stimulus (Marsl et al , 1969), and to be independent of associa tive or conditioning processes, inhibition ma be found on the first trial on which the startl stimulus is preceded by a pulse of noise (Krau ter et al , 1973)

The inhibitory process also proved to b rather sensitive, inhibition appearing in the ra when a 35-dB SPL noise burst precedes the in tense startle eliciting stimulus by 150 mse (Hoffman & Wible, 1970) This sensitivity ha led to speculation that a comparable paradigr might have an application in audiometry an modification of the reflex by an appropriat prestimulus would verify the patient s acuit for tones of that frequency and intensity It vestigation of this possibility was at first fru trated for two reasons Inhibition was at th time believed to be specific to the acoust startle reflex, and the use of intense startl stimuli with humans would have been inappr priate The second obstacle was the mea urement of the startle reaction This respons is easily measured with a stabilimeter whe small animals are used, but more sophisticate

The work was supported by Grant MH24044 from t National Institute of Mental Health directed by Howa S Hoffman

dures such as electromyography, would ... d with humans

Recent research has shown that reflex inhibition might be a feasible audiometric procedure after all. First came the discovery that the startle-eliciting stimulus and the inhibitory stimulus need not be of the same sensory modality. The acoustic startle reflex of the rat is inhibited by a visual prestimulus, and the ... s visually elicited startle is inhibited by auditory prestimulation (Buckland et al., 1969, ... et al. 1976). These studies expanded the ... of available eliciting stimuli by demonstrating that inhibition by auditory prestimulation ... result simply from an interaction with a subsequent auditory eliciting stimulus. More encouragement came from the observation that prestimulation effects can be found in the human when only the eyeblink is measured instead of the skeletal startle response (Graham 1975). Furthermore, inhibition of the reflexive eyeblink by acoustic stimulation has been demonstrated when the eyeblink itself was elicited by a gentle puff of air (Krauter et al. 1973). These were particularly important revelations because the eyeblink can be safely and objectively measured, as psychologists have done for years in the course of classical conditioning studies, and because tactile stimulation such as that used by Krauter et al. would certainly be more appropriate than intense auditory reflex eliciting stimuli for evaluating the hearing impaired patient.

These observations have stimulated additional research with humans which laid the groundwork for the research reported here. Those studies confirmed the sensitivity of the inhibitory process—inhibition was demonstrated with a 30-dB SL tone burst preceding a tactile eyeblink eliciting stimulus—and demonstrated that the amount of inhibition found with a tone burst of fixed intensity was independent of the intensity of the eliciting stimulus, i.e., the antecedent tone reduced the response by a constant amount which was independent of the baseline reponse amplitude (Marsh 1976). These data showed that the procedure could be readily administered at least with adults, and that great attention to the intensity of the reflex-eliciting stimulus, a puff of air striking the face lateral to the eye, is unnecessary. In spite of these encouraging results, reflex inhibition still did not appear to be entirely satisfactory. The inhibitory effect of weak tones, while statistically demonstrable, was not great, and certain subjects failed to show inhibition at all.

At this point reflex inhibition by monotic prestimulation was attempted in contrast to all previous research where inhibitory stimuli were presented binaurally in a sound field or by earphones. Contrary to expectation monotic prestimuli inhibited the eyeblink reflex to a much greater extent than did binaural stimuli (Marsh et al. 1976). Even those subjects who had failed to show any inhibition before, inhibited quite reliably when retested with monotic inhibitory stimuli. The same study examined the symmetry of reflex inhibition by testing all left right combinations of auditory prestimulus, tactile eliciting stimulus and response tranducer. No interaction was found between ear under test and either of the other two variables, implying that the reflex-eliciting stimulus delivery device and response transducer may each be arbitrarily applied to one side of the face or the other without regard to which ear is to be tested.

EXPERIMENT 1

This study served to integrate the results of the above cited experiments and to provide an indication of the potential performance of reflex inhibition audiometry in clearing comparable populations of suspicion of hearing loss. So that this study would better address itself to clinical issues, tone intensities were set with reference to ANSI norms rather than being specified as sensation levels; also a range of audiologically significant frequencies were examined, rather than the single 1 kHz tone employed in other human research in this laboratory.

Method

Subjects Twelve graduate and undergraduate students at Bryn Mawr College served in this experiment

Apparatus The apparatus typically used in experimental investigations employing the eyeblink, as in classical conditioning research, does not lend itself well to the ultimate goal of clinical applications where cost, portability, and patients' comfort are important considerations For this reason equipment which meets these requirements was designed for the research reported here

The device for generating the eyeblink-eliciting airpuff was an air suspension acoustic speaker modified to produce a highly reliable airpuff The diaphragm of the speaker was coated with silicone rubber and the speaker was enclosed in such a way that displacement of the diaphragm forced air through a tube leading to the subject The airpuff was generated by charging a bank of capacitors (400 μF) to 200 V, then discharging them through the modified speaker by means of a silicon controlled rectifier with a 1-henry inductance in series to filter out audio frequency components from the current surge An 8 ohm attenuator was constructed to permit adjustment of the airpuff intensity without affecting the load experienced by the solid state capacitor-discharge circuit

The eyeblink was measured with a modified d'Arsonval meter fastened to the end of the air delivery tube The pointer on the meter was extended with a length of polyethylene tubing which was taped to the eyelid This unit translated movements of the eyelid into rotation of the meter coil in a magnetic field and generated a voltage proportional to the velocity of the motion This voltage was amplified, rectified, and measured by a digital voltmeter with storage capability

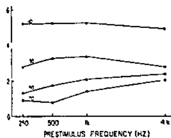
To simplify preparation of the subject, the air delivery tube response transducer assembly was attached directly to the earphone headset used for the delivery of acoustic signals A specially designed clamp permitted rapid adjustment of this assembly so that th airpuff struck the face just lateral to the eye

The tones which served as anteceder stimuli were generated by controlling the ou put of a Hewlett-Packard Model 200AV audi oscillator with a Grason Stadler electron switch (Model 829 D) A General Radio d cade attenuator (Type 1450-TB) permitt justment of tones with reference to A (1969) norms, and a Daven A-1023-G a uator permitted independent adjustment stimuli in 2-dB steps Auditory stimuli we delivered through TDH 39 earphones fit with MX-41/AR cushions The earphones test stimuli were calibrated with a C Radio precision sound level meter (M 1561-A), fitted with a P 7 microphone ANSI Type 1 coupler, using ANSI (1 norms

Grason-Stadler Model 471-1 interval controlled the duration of prestimuli as w as their lead time, measured as the interval b tween arrival of the leading edge of the stimulus and the leading edge of the airpu and taking into account delays involved in transmission of the airpuff through the tub from its generator to the subject solid state and relay circuitry operated puff generator under the control of the timers

All studies were conducted in an I double wall sound treated room having ambient noise level below 25 dBA This c ber was suitably furnished and lighted equipped with a rear projection screen so 35 mm slides could be projected from the c trol room A closed-circuit television cam and an intercom permitted continuous toring of the subject

Procedure After each subject had appraised of the nature of the research an agreed to participate, he or she was the chamber and fitted with the head ing earphones airpuff delivery tube response transducer The subject's thres for the tones employed in this study were determined by the method of Carhart & J



[Fig] 1 Response amplitudes to airpuff stimulation with [pre]stimuli of several audiologically significant intensities [in]dicated as the parameter in dB HL) and frequencies [Th]e inaudible −10-dB HL prestimuli provide a basis for [as]sessing the inhibitory effects of the other prestimuli

[1]959) this clinical technique being modified [so] that test tones were varied in increments [of] 2 dB rather than 5 dB

During the experimental session itself the [de]sired combinations of antecedent auditory [st]imulus and blink eliciting airpuff were [p]resented at 30-sec intervals To combat bore[do]m 35 mm color slides of paintings or nature [su]bjects (wild animals, scenic views, etc) [w]ere shown to the subject on the rear projec[ti]on screen in the chamber Except for a brief [in]terval between trials when slides were [c]hanged, they were projected continuously [E]xperimental sessions, including briefing and [p]reparation of the subject, required no more [t]han 30 minutes to complete

Each subject was evaluated for one ear [o]nly half for the right and half for the left [P]restimuli having frequencies of 250, 500, 1000 and 4 000 Hz were presented, each at [i]ntensities of −10 30, 50, and 70 dB HL, each frequency intensity combination being pres[e]nted eight times over the course of two ses[si]ons Every prestimulus had a rise time of 10 [m]sec a 200-msec duration, and an onset pre[ce]ding the airpuff by 100 msec The rise time employed here although less than that speci[fi]ed for clinical audiometers, had been found to be somewhat more effective than longer rise times in inhibiting the eyeblink reflex (Marsh, 1976)

Results and Discussion

Examination of the averaged responses (Fig 1) shows prestimuli of 30 dB HL to strongly inhibit the eyeblink reflex, relative to the subthreshold −10-dB condition, for all frequencies tested A two way repeated measures analysis of variance confirmed a significant effect of prestimulus intensity, $F(3\ 33)=115\ 58$ $p<0\ 01$ Although no effect of frequency was demonstrated, $F(3, 33)=1\ 96$ n s a significant frequency-intensity interaction was found, $F(9, 99)=2\ 76$, $p<0\ 01$

Scores of individual subjects are naturally of more interest to the clinician than are group averages Each subject had smaller average responses at 30 dB HL than at −10 dB for every frequency except for a single subject at a single frequency, she did show substantial inhibition by the 50-dB prestimulus at that frequency It must be admitted that inhibition by 30-dB prestimuli was not so strong for every subject as to be clearly discriminated from random variation but it should also be remembered that this study only employed eight observations per stimulus condition Moreover, if a one volt response decrement is arbitrarily taken to be evidence of inhibition the data remain encouraging Seven subjects met this criterion for all frequencies at 30 dB and all did for every frequency at 50 dB except for two subjects who failed to reach this criterion at 50 dB but did so at 70 dB for a single frequency, even they met the requirement at 50 dB for three frequencies

EXPERIMENT 2

Although research with adults yielded encouraging evidence in support of the potential of reflex inhibition audiometry it provided no assurance that the technique would be effective with infants and young children in the age range where objective techniques are most needed Because of this consideration the reflex inhibition procedure was applied to a number of children ranging from 6 weeks to 5 years of age These studies were less rigorous in design and execution than the adult research, having the primary objective of providing assurance that reflex inhibition could be found throughout this age range

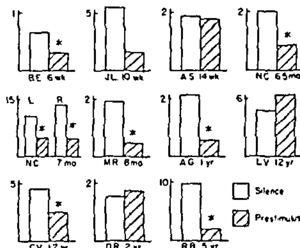


Fig 2 Individual reflex amplitudes in infants and children measured in silence and with a 1 kHz 70-dB HL prestimulus An asterisk indicates significant response inhibition by the prestimulus ($p<0.05$)

Method

Subjects Eleven babies and children participated in this study All except one, the daughter of a college employee, were recruited for this work by their family physician None were suspected of any hearing impairment

Apparatus It proved desirable to modify the equipment used with adults to make it more suitable for infants It was found advantageous to attach the response transducer directly to the infant's forehead The face of the meter housing was secured to the forehead above the eye with adhesive material of the kind used to attach ostomy appliances A strut constructed of polyethylene tubing linked the meter's needle to the eyelid, where it was attached with a small strip of Micropore tape This device which weighs less than 14 gm and is quite small (approx 2×4×4 cm) has proved to be easy to apply and comfortable for the infant The eyelid attachment linkage is quite safe and well tolerated being of soft material with smooth edges

The airpuff delivery tube was connected to a hand held unit which was placed about 3 cm in front of the infant's eye during each trial This handpiece was constructed from a soft plastic pacifier and presented no hard surfaces which might injure the baby The shape of the device, with its soft eyeshield (the nipple of the pacifier having been removed) further protected the infant as it presented a large flat surface which could not enter the eye The acoustic test stimuli were delivered to the baby via a single earphone (TDH39 fitted with an MX41 AR cushion) that was held against the baby's ear at the start of each trial A pushbutton switch on the handpiece of the air delivery system permitted the tester to initiate a trial when the handpiece and earphone were correctly positioned

Procedure During a test session the subject sat on its parent's lap One experimenter in the control room selected the stimulus configuration to be employed and the other experimenter, who was with the infant, was notified by means of a signal light when a trial was to begin He then positioned the earphone and airpuff delivery handpiece and initiated the trial This experimenter had no information as to the stimulus employed on a particular trial or the amplitude of the infant's eyeblink

Ten airpuffs were presented in silence interspersed with ten airpuffs preceded by a 70 dB HL, 1-kHz tone, usually having a 100 msec lead time, although 200 msec was used with several subjects As in Experiment 1, the tone had a 10 msec rise time and continued for 100 msec after the airpuff All subjects were tested with one ear only, except for NC who returned for a second session in which both ears were tested The procedure proved to be comfortable for the babies and children who gave no evidence of annoyance during the test

Results and Discussion

The data for each subject are shown in Fig 2 An asterisk indicates that the responses in the prestimulus and stimulus conditions were significantly different according to a two tailed t-test on that subject's data, $p<0.05$ The successful demonstrations of reflex inhibition cover the entire age range As seen in Fig 2 several subjects did not exhibit reliable differences between conditions It must be re

ognized however, that the present work represented only an initial effort It seems possible that in future work such failures could be prevented by a choice of stimulus parameters more appropriate to this young population

GENERAL DISCUSSION

Although a declaration of the efficacy of reflex inhibition audiometry would be premature this technique does show great promise The procedures involved have proven themselves simple to administer and safe and comfortable for the subject or patient The investigation with infants was especially encouraging in demonstrating the ease with which even such young subjects can be tested and confirming their lack of irritation by the apparatus or stimuli Reflex inhibition commends itself also in requiring relatively inexpensive equipment compared to that required for certain other objective procedures and, if the adult research is any indication, sufficient sensitivity to clear many patients of suspicion of even a moderate hearing loss

Caution dictates of course that no diagnosis of hearing loss should be based on reflex inhibition audiometry until thorough clinical validation of the technique has been completed The preliminary work with infants certainly supports such a caveat Although some subjects of every age showed inhibition others did not and additional research is needed to identify the factors responsible for this variability

Relatively little clinical evaluation of the procedure would be required, however, to permit its use as a screening test with infants since the presence of inhibition with a given test stimulus is strong evidence that the tone is above the patient s threshold Used in this way reflex inhibition audiometry would seem to have great potential in clearing young patients so that only those whose responses were inconclusive would be subject to more time consuming or expensive test procedures Reflex inhibition audiometry might be valuable too as a confirmation of audiograms determined by other means, a rapid test at one or two frequencies would lend confidence to the previous determination if good agreement is found

ACKNOWLEDGEMENT

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ZUSAMMENFASSUNG

Das reflexive Blinken des Auges das bei den Taststimulant hervorgerufen ist wird gehemmt wenn ein akustischer Stimulant den auslösenden Stimulant um 0 1 Sekunde vorangeht Bei erwachsenen Versuchspersonen ist die Reizschwelle fur diesen Effekt sehr gering und weist darauf hin daß dieser hemmende Reflex für die Audiometrie verwendet kann Eine einleitende Untersuchung mit 6wochigen und alteren Sauglingen und Kindern weist ebenfalls auf diese hemmenden Reflexe hin

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EXTRA INTERNAL HAIR CELLS

A Scanning Electron Microscopic Study

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Abstract The extra internal hair cell (EIHC) of the human cochlea was observed by means of a scanning electron microscope The EIHC was found not infrequently in all turns of the human cochlea It was located medial to the IHC row The inner pillar cells showed an abnormal structure The anatomical relationships between the displaced IHC and EIHC and the inner pillar cell were classified into five types The origin of these anomalies is discussed from an embryological viewpoint

It has been said that the internal hair cells show less abnormality than the external hair " in regard to arrangements of cells as well , morphology However, extra internal hair cells (EIHC) are not infrequently observed when examining the surface of the organ of Corti under the scanning electron microscope (SEM) As early as 1881, Retzius already reported on this and named this phenomenon "überschussige innere Haarzellen" Kolmer described a similar finding in a light microscopic study (Fig 1)

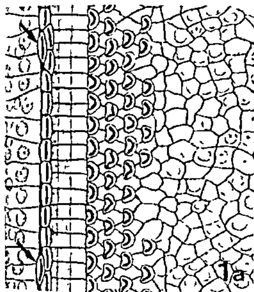
While surveying the surface of the human organ of Corti, we have found that abnormal shape and arrangement of the supporting cells such as the inner pillar cells and border cells are observed in the organ of Corti adjacent to EIHC The purpose of this paper is to describe the morphology of EIHC and to classify cell arrangements of the extra internal hair cells and the supporting cells and to review the available literature

MATERIAL AND METHOD

Temporal bones from aged people of over 8(years old who died of pneumonia and hear diseases were used in this study The ' were removed at autopsy about one hour post mortem and fixed in 10% formalin solution The membranous labyrinth was removed from the temporal bone and, was then fixed in 2.5% glutaraldehyde solution (0 2 M phosphate buffer) at pH 7 4 for 12–24 hours Subsequently the materials were fixed with 2% tannic acid solution and 2% osmic acid solution according to Murakami's method Specimens were dried using the critical point drying method after dehydration through graded alcohol and amyl acetate They were then coated with gold palladium in a vacuum evaporator (ion sputtering) The specimens were observed under a scanning electron microscope (JSM S type)

RESULTS

The internal hair cells (IHC) form a single row in the organ of Corti In the aged the external hair cells showed a loss of stereocilia whereas this loss was much less marked in the IHC Instead, extra cells which lie outside of the usual single row were found in the IHC The EIHC usually exists medial (toward the modiolus) to the normal position of the IHC row—never on the outside of it Under low power viewing



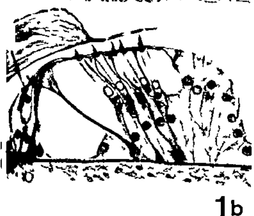


Fig 1 (a) An extra internal hair cell In 1881 Retzius drew a similar duplication of an internal hair cell and termed it uberschussige innere Haarzellen (*arrow*) (b) In 1977 Kolmer described a similar finding in a tangential section of the spiral organ with a light microscopy (*arrow*)



Fig 2 A low magnification SEM picture of the surface of spiral organ An extra internal hair cell (*arrow*) exists medial (toward the modiolus) to the normal position of the internal hair cell row (*IHC*) The internal hair cell row forms a convex curve laterally (toward the external hair cell (*OHC*)) ×800

the IHC row formed a convex curve laterally at the point where the extra cell existed (Fig 2) There was no predominance of the localization of the EIHC in any part of the cochlea They were found in the upper, middle and the basal turns of the human cochlea

Under a high power view, no particular structure was found in the cuticular plate and stereocilia in these EIHCs However, the supporting cells around the EIHC showed unusual cell arrangements

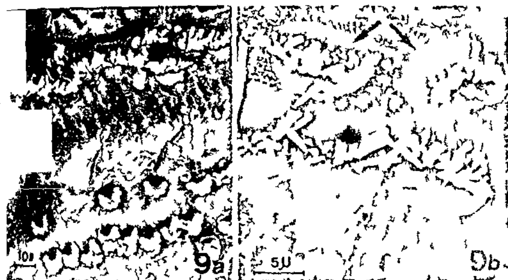
Fig 3 indicates the normal arrangement of the IHC and supporting cells The cuticular plate of the IHC is elliptical and it protrudes a bit further than the free surface of the surrounding supporting cells The long axis of the elliptical cuticular plate lies parallel with the row of IHC

Lateral to the IHC there is a head of the inner pillar cells Its head shows a rectangular shape, the long axis of which is perpendicular to the row of IHC It is tightly in contact with the cuticular plate of the IHC

Medial to the IHC there is a border cell which has microvilli on its surface The border cell extends partly into the intercellular space between IHCs Therefore the border cell and the head of the inner pillar cell contact each other

As the terminal web was poorly developed in the border cell, its cell margin was not clear The shape and size of the border cell is, therefore, obscure Medial to the border cells is the inner sulcus cell the free surface of which has no microvilli


BC
IPC
3
5μ
5μ
BC
7
IPC
5μ
5μ



9 Ex ra n ernal hair cells are located closely (*a ar* o ide by s de (*b a ow*) *a* ×800 *b* ×2400

The anatomical relation of the EIHCs and pporting cells can be classified as follows

The type A (Figs 4 and 10*A*) This is a dis cement of IHC rather than EIHC A dis aced IHC is seemingly pushed medially from e normal IHC row by a head of the inner pil r cell

Type B (Figs 5 and 10*B*) There is a gap in the IHC row However tl e h id of the inner pillar cell is in normal pos t on This IHC is medial to the IHC row The border cells with numerous microvilli surround the IHC In other words this type B has no extra IHC but rather a dislocation of an IHC The original area is replaced by the border cell

Type C (Figs 6 and 10*C*) The IHCs lie side by side tightly An EIHC is medial to and close to the IHC row

Type D (Figs 7 and 10*D*) Like Type C there is no deficit of an IHC and in the IHC row However an EHIC is present medial to and away from the IHC row The border cell with numerous microvilli is between the EIHC and the IHC row

Type E (Figs 8 and 10*E*) This type shows a mixture of types A and D An EIHC is lo cated med al to and close to the IHC row The head of the inner p llar cell passing through the IHC row is in contact with the EIHC s cuticular plate The head of the inner pillar cell is not rectangular but shows a curved shape

The EIHCs are observed at times located side by side or closely (Figs 9*a b*) A scheme of the above classification is shown in Fig 10

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ig 3-8 *H* gh magnif cat on SEM p ctures of normal and xtra nternal hai cel s

ig 3 how the normal arrangement of an nternal ha r ell and he support ng ce ls An nternal hair cell (*IHC*) ocated be ween the head of nternal p llar cells (*IPC*) and orde ce ls (*BC*) ×2400

ig 4 hows an ex ra n e nal ha r cell correspond ng to n Fig 10 An extra n ernal ce l (*a ro*) moves toward he modio us w h the head of the nternal p llar cells *IPC* × 400

F g 5 hows an extra n ... n F g 1 ... ounded by ... l The he ... end toward

F g 6 shows an ex ra n ernal ha cell (*a ro*) located n ide the row of internal hai cel s ×2400

F g 7 hows a s milar f nd ng to F g 6 An extra n ernal ce (*arr*) does no come n o contact wi h ord nary n ernal ha ce and s surrounded by the border cells (*BC*) 500

F g 8 shows an ex ra n ernal hair cell (*a o*) corre spond ng o (*E*) n F g 10 Th s type appea s to be a m x ture of types (*A*) and (*D*) n F g 10 *IPC* head of n ernal pillar cell × 400

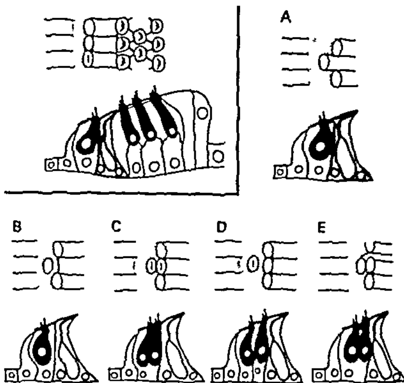


Fig 10 A schematic drawing of the classification of the extra internal hair cells

DISCUSSION

Little has been mentioned about the extra internal hair cell (EIHC) since the first description of Retzius and later Kolmer Recently Soudijn described briefly the EIHC of the guinea pig using the SEM The paucity of the literature is mainly attributable to a low incidence of EIHC (Kolmer, 1927, Engstrom et al , 1970)

The EIHC exists in the organ of Corti of man (Retzius, 1881 Kolmer, 1927) rabbit (Retzius 1881) and guinea pig (Soudijn, 1976) It is also found in rat, mouse and dog Therefore, it is reasonable to assume that the EIHC is present in the organ of Corti of many species and not specific to any one species It is our impression that there are more EIHCs in the human organ of Corti than in other animals

As to the location of the EIHCs Soudijn reported that they are found more in the upper turn of the guinea pig Kolmer s specimen was also from the upper turn of the cochlea In the present investigation, the EIHCs were found more in the apical turn of the human organ o Corti, though they were also widely distrib uted in the middle and basal turns

The cytoarchitecture of the IHC and sup porting cells was investigated in detail b transmission electron microscopy (Iurato 1967, Kimura et al , 1965, Kimura 1975) According to Kimura (1975), the supporting cell of the IHC are the inner pillar cell the inne phalangeal cell, the border cell and the inne sulcus cell The inner phalangeal cell is locate between the inner pillar cell and the IHC It i a small cell, irregularly shaped and havin many microvilli on the free surface The bor der cell is located between the IHC and th inner sulcus cell

On observing the fine surface of the huma organ of Corti by means of SEM the inner pil lar cells and the inner sulcus cells were easil distinguishable whereas the inner phalangea cells and the border cells were hardly distin guishable The microvilli observed in the nar row space between the IHCs is possibly o the free surface of the inner phalangeal cell

found it difficult to distinguish the inner langeal cell from the border cell even when was a relatively wide space between the IHCs (Fig 5) the border cell is defined as having numerous microvilli on its free surface

In this paper we propose to classify the arrangements of EIHC and supporting cells into five types The head of the inner pillar cell displayed an abnormal shape, whereas the border cells of the inner sulcus cell do not show any anomaly Considering combinations of the presence or absence of abnormality of the IHC row and head of the inner pillar cell, the above mentioned five types can be grouped as follows

Group I There is no gap in the IHC row As the EIHCs lie medial (toward the modiolus) to the normal IHC row, the inner pillar cells show no anomaly

Group II There is a gap in the IHC row No abnormality in the head of the inner pillar cell

Group III There is a gap in the IHC row The pillar head extends toward the modiolus

Retzius observed the EIHC in a newborn infant Soudyn (1976) thought the EIHC to be a normal variation Bredberg (1968) found the EIHC in a 6-month old foetus The cells in the organ of Corti are quite regularly arranged in the foetus After birth, hair cells show an irregular arrangement However, we are of the opinion that the EIHC observed in aged persons in the present study are formed during the development of the inner ear It is still a matter of dispute as to which turn of the cochlea develops first (Pujol & Marty, 1970) However, studies of Kolmer (1927), Kollmann (1898) and Tonot (1894) have been generally accepted in regard to the development of the organ of Corti. According to Kolmer, differentiation of the organ of Corti begins as early as the 3rd foetal month in the epithelial mound of the cochlear duct The organ of Corti develops rapidly from the 3rd to 4th foetal month Thickened epithelium becomes the neuroepithelium Three rows of the external hair cells are differentiated from a lateral part of the neuroepithelium whereas one row of IHC results from a medial part

In the 7th month the inner sulcus is formed at the same time, and a space is built between the IHC and the external hair cells This develops into the tunnel of Corti which is formed by the inner and outer pillar cells

The hair cell is differentiated and completed from the neuroepithelium prior to the formation of the pillar cell Considering the various arrangements of EIHC from the organogenesis of the organ of Corti, anomaly of cell arrangement occurs in the early stage of development in Group I The EIHC with anomaly of head of the inner pillar cell observed in Group III seems to arise in a relatively late stage of development

The function of the EIHC is unknown Since the IHC is innervated much more than the external hair cell in the human organ of Corti (Nomura 1976) it is important to study whether or not the EIHC has a pattern of innervation similar to that of the IHC

ZUSAMMENFASSUNG

Die überschüssigen inneren Haarzellen der Schnecken von Menschen wurden mit dem Raster Elektronenmikroskop betrachtet Diese Zellen befinden sich auf der ganzen Windung der Schnecke und zwar innerhalb der inneren Haarzellen Reihe Auch an den nebenliegenden inneren Pfeilerzellen wurden Anomalien beobachtet Die anatomischen Beziehungen zwischen den verschobenen inneren Haarzellen überschüssigen inneren Haarzellen und inneren Pfeilerzellen wurden in 5 Typen geteilt und die Bildung dieser Anomalien wurde aus den genetischen Ansichten besprochen

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REVERSIBLE AND IRREVERSIBLE CHANGES OF THE STRIA VASCULARIS

An Evaluation of the Effects of Ethacrynic Acid Separately and in Combination with Atoxyl

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Abstract The morphological changes in the cochlea following administration of ethacrynic acid occur initially in the stria vascularis of the basal coils as an increased extracellular vesiculation of the marginal cells followed by inter and intracellular oedema in the intermediate cell layer The combined administration of ethacrynic acid and atoxyl (individual doses) can cause irreversible damage to the cochlear hair cells and the stria vascularis while the administration of each of them separately in the same low dose did not cause hair cell degeneration or permanent morphological changes of the stria vascularis An increased penetration of atoxyl into the cochlea is likely to occur due to the ethacrynic acid induced change in the permeability of the endolymphatic partition so that the earlier known penetration of atoxyl into the cochlea is increased

The stria vascularis of the inner ear is of fundamental importance for the preservation of the specific composition of the endolymph, indicating a close interaction between the hair cells of the cochlea and the stria vascularis In recent years the increase in the use of potent diuretics such as ethacrynic acid, furosemide and others in clinical work has, because of ototoxic adverse effects, focused attention to the close relationship that exists between the kidney tubules and the stria vascularis of the inner ear

Morphological studies from ethacrynic acid treated specimens have revealed a more pronounced and earlier damage in the stria vascularis than of the hair cells (Matz et al , 1969 Quick & Duvall, 1970) as also described following furosemide administration, another potent diuretic (Quick & Hoppe 1975) Brummett et al (1977) correlated the physiological alterations of the cochlear potentials with the ultrastructural changes in the stria vascularis during the early stages of ethacrynic acid influence on the inner ear These changes appear to be greatly reversible as compared with the effects of atoxyl an arsenical compound, which can cause irreversible damage of strial cells (Anniko & Wersall 1975 Anniko 1976*a*)

A combination of two potentially ototoxic drugs, e g diuretics and aminoglycoside antibiotics, when needed may involve considerable difficulties in their clinical use The present study elucidates morphologically the mechanisms at work in the interaction between a diuretic ethacrynic acid and another type of ototoxic substance (non diuretic) in this case atoxyl in their combined effect on labyrinthine structures

MATERIALS AND METHODS

Sixty one healthy young guinea pigs (200–300 g) without clinical evidence of otitis media and exhibiting a normal Preyer's reflex were used for the experiment Thirty of these animals were injected with ethacrynic acid (Edecrina®,

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Table I *Table illustrating Preyer's reflex change in a number of guinea pigs of the experimental group initially receiving only ethacrynic acid (EA) according to the dose*

Animal no	EA (mg/kg)	Preyer s reflex (hours) 0	1	2	4	6	12	24	48	72	96	Investigated (hours after injection)
3	25	+	+	+	+	+						6
13	35	+	+	+	(+)	(+)						6
15	35	+	+	(+)	(+)	(+)						6
9	70	+	+	(+)	−	−						6
55	25	+	+	+	+	+	+	+[a]	+[a]	+[a]		7 days
56	25	+	+	+	+	+	+	+[a]	+[a]	+[a]		7 days
51	50	+	−	−	−	(+)	+	+[b]	(+)	(+)	(+)	4 days
52	50	+	−	−	−	+	+	+[b]	−	−	(+)	4 days
31	100	+	−	−	−	−	−	(+)	+			2 days
41	100	+	−	−	−	−	−	−	+	+	+	4 days
39	200	+	−	−	−	−	−	(+)	+			2 days

[a] Injection of 25 mg/kg of ethacrynic acid
[b] Injection of 70 mg/kg of atoxyl

MSD) and 31 were treated with a combination of ethacrynic acid and atoxyl (Pro Gen® Sodium, Abbott Laboratories Ltd) The control group consisted of 5 untreated guinea pigs

The animals treated with ethacrynic acid only were given a 1% solution of ethacrynic acid in isotonic saline solution subcutaneously (s c) as a single dose, 10–200 mg/kg b w The presence or the absence of Preyer's reflex was recorded every hour during the first 12 hours and thereafter every sixth hour until sacrificing the animal (Table I)

Guinea pigs treated with a combination of ethacrynic acid and atoxyl, a 2% solution in sterile water were injected s c with atoxyl (50–70 mg/kg b w) either before simultaneously with, or following the administration of ethacrynic acid (Tables III III IV)

Following decapitation of the animal the labyrinth was perfused with 2% osmic acid a fixative for 2 hours, rinsed in sodium phosphate buffer (pH 7 2–7 4) whereafter the microdissection was performed to separate the cochlea from the rest of the labyrinth The specimens were dehydrated in increasing concentrations of alcohol and embedded in Epon Light microscopic sections were stained with toluidine blue, whereas the ultrathin sections for electron microscopy were stained with uranyl acetate and lead citrate

Table II *Table illustrating Preyer s reflex change in a number of guinea pigs following administration of ethacrynic acid and atoxyl at the same point in time*

Animal no	EA (mg/kg)	A (mg/kg)	Preyer s reflex (hours) 0	1	2	4	6	12	24	48	72	96	Investigated (hours after injection)
25	35	70	+	+	−	−							4
26	35	70	+	+	+	+							4
35	100	100	+	−	+	+							4
38	100	100	+			−	−	−	(+)				24
40	100	100	+			−	−	−	−	−			48
53	50	70	+		−	−	−	−	−	−	−	−	24
54	50	70	+		−	−	−	−	−	−	−	−	96
57	25	70		+	+	+	+	+	+[a]	−			48
58	25	70		+	+	+	+	(+)	−	−	−		72
61	25	50		+	+	+	+	(+)	(+)	(+)			48

[a] Renewed injection of 25 mg/kg of eth ı rynic acid

Table III. *Table illustrating Preyer's reflex change in a number of guinea pigs following administration of ethacrynic acid 1–2 days before the injection of atoxyl*

Animal	EA (mg/kg)	A (mg/kg)	Preyer's reflex (hours) 0	1	2	6	12	24	48	72	96
18	25	70	+	+	+	+	+	+[a]	+	+	+[b]
19	25	70	+	+	+	+	+	+	+	+	+[b]
1	50	70	+	–	–	(+)	+	+[a]	(+)	(+)	(+)[b]
2	50	70	+	–	–	(+)	+	+[a]	–	–	(+)[b]
3	50	70	+	–	–	–	+	+[a]	(+)	(+)	[b]

[a] Injection of atoxyl
[b] Time of sacrifice

RESULTS

Ethacrynic Acid

Morphological changes of the inner ear were confined to the stria vascularis and the outer hair cells without involving the neighbouring spiral ligament, spiral prominence, or Reissner's membrane. The structural changes were extensive in the animals which received the highest doses, exceeding 60–70 mg/kg, and these started in the basal part of the cochlea.

An amount of 35 mg/kg (single dose) did not cause any structural alteration in the stria vascularis during the first 2 hours after its administration although Preyer's reflex became impaired during this time. In specimens studied 4–6 hours after the administration of the ethacrynic acid, a mild intercellular oedema could sometimes be observed in the intermediate cell layer of the stria vascularis of the basal coil. A normal ultrastructure was present in specimens treated with a dose less than 35 mg/kg (subcutaneously) and also in those which were investigated 2–7 days following the administration of 10–60 mg/kg.

With an increasing amount of ethacrynic acid (≥70 mg/kg) strial changes were also observed when the specimens were investigated only ½–1 hour following the subcutaneous injection. The changes after this time were usually restricted to the 1st and the 2nd coils but 2–4 hours following the ethacrynic acid administration all coils were morphologically altered. Specimens investigated 24–48 hours after the injection with the same amount of ethacrynic acid showed less extensive changes in the stria vascularis than those observed after only 2–5 hours, thus indicating a reversible process.

The structural changes of the stria vascularis followed the same basic pattern in all investigated specimens. Initially an increased thickness was observed in the light microscope. The electron microscope showed a

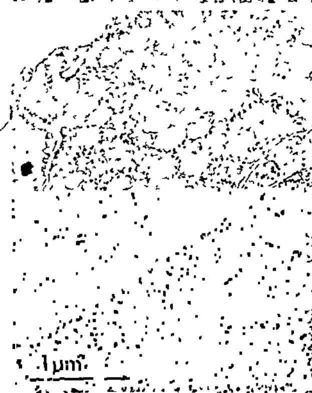
Table IV. *Table illustrating Preyer's reflex change in a number of guinea pigs following administration of ethacrynic acid 3–24 hours after the injection of atoxyl*

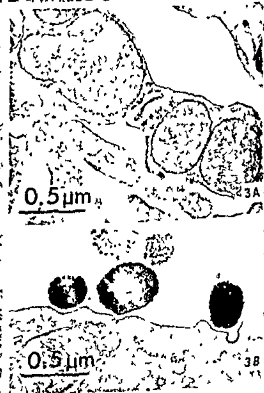
Animal no.	EA (mg/kg)	A (mg/kg)	Preyer's reflex (hours) 0	1	2	6	12	24	48	72	96
21	35	70	+	+	+[a]	+	+	+[b]			
22	35	70	+	+	+[a]	(+)	(+)	+[b]			
44	50	100	+	+	+	+	+	+[a]	–[b]		
45	50	100	+	+	+	+	+	+[a]	(+)	+	+[b]
46	50	100	+	+	+	+	+	+[a]	(+)[b]		

[a] Administration of ethacrynic acid
[b] Time of sacrifice


1µm
1A


1µm
1B




0,5µm
3A


1µm


0,5µm
3B

mild intercellular oedema in the intermediate cell layer, beginning around the strial blood vessels (Fig 1) The marginal cells contained an increased number of cytoplasmic vesicles (Fig 2) but also the mitochondria were affected early showing a slight swelling and dissolution of their internal structure (Fig 3) Similar mitochondrial changes were also found in the intermediate cells Later, an increase of the intercellular spaces and a vacuolization of both the marginal and the intermediate cells occurred However, the marginal cells were less affected than the intermediate cells, although both cell types revealed a shrinkage of the cell volume during the late stages of strial changes (Fig 4) The melanin granules normally contained within the intermediate cells were resistant to atrophy However, melanin particles were often found in the endolymphatic space and many of them were in contact with the surface of the marginal cells and formed imprints in the cytoplasm (Fig 3b) as if they had been ejected from the marginal cells

No morphological changes were discernible in the capillary endothelium of the blood vessels and the basal cells always remained normal In spite of the sometimes pronounced changes of the stria vascularis (Fig 4) the lining of the marginal cells facing the endolymphatic space always remained smooth without disrupting the junctions between the cells The morphological changes of the stria vascularis appeared greatly reversible Alterations registered 24–48 hours following the treatment were less pronounced than those observed only a few hours following the ethacrynic acid injection The organ of Corti appeared intact even when corresponding parts of the stria vascularis were morphologically changed

Fig 1 Electron m ... Increased number of cytoplasmic ... in a marginal cell

Fig 2 EM Stria vascularis Early stage of ethacrynic ... induced changes (A) In the marginal and the inter... cell extensions mitochondria often reveal a slight ... of the cristae An oedema is present between ... cells (B) Melanin particles in close contact with the marginal cell surface as if they were expelled into the scala media

Combinations of Ethacrynic Acid and Atoxyl

Administration of ethacrynic acid and atoxyl simultaneously

The morphological changes were closely correlated to the change in Preyer's reflex (impairment or total loss) No considerable structural alteration of the stria vascularis was observed in animals with a preserved Preyer's reflex

Simultaneous administration of ethacrynic acid (≥50 mg/kg) and atoxyl (70 mg/kg) caused a rapid and mostly a persistent loss of Preyer's reflex The morphological changes of the stria vascularis were however frequently less pronounced than those following only ethacrynic acid treatment after the same length of time, except for the rejection of some strial cells from the surface of the stria vascularis mainly close to the spiral prominence (Fig 5a–b) In these specimens only a mild intercellular oedema occurred Most rejected cells were recognized as marginal cells showing vesiculation of the cytoplasm but containing many quite well preserved mitochondria The hair cells of the organ of Corti were in these specimens ultrastructurally normal at the adjacent level of the stria vascularis Afferent nerve endings could appear swollen but efferent nerve endings were morphologically unchanged

The combined administration of ethacrynic acid and atoxyl often resulted in a prominent change in the mitochondrial features, showing osmiophilic inclusion bodies adjacent to the mitochondrial cristae These inclusions were found both in mitochondria with a preservation of cristae and in those in various stages of degeneration with fragmentation of the internal structure (Fig 6)

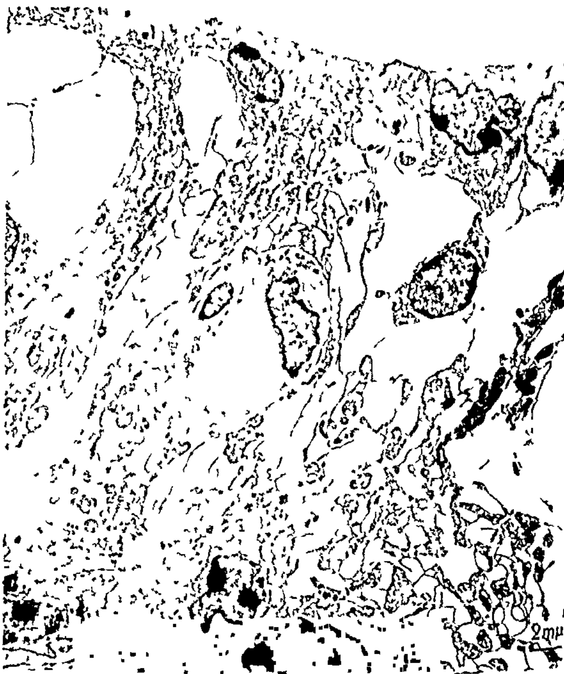


Fig 4 EM Severe oedema of the marg nal and inter mediate cell layers of the stria vascularis following the admin strat on of ethacrynic ac d The basal cells appear morphologically intact The marg nal cell cytoplasm tains a great number of small ves cles

Fig 5 EM Stria vascular s Comb nat on of ethacryn c ac d and atoxyl Damaged strial cells are expelled into the endolymphat c space

Fig 6 EM Stria vascularis Comb nat on of ethacryn c ac d and atoxyl Marginal cell Osmioph l c inclus on bod es (*arro vs*) are frequently found n m tochondria in rather well morpholog cally preserved cells

Fig 7 EM Comb nat on of ethacryn c ac d and a Stria vascularis Osm oph l c debris n the region bet intermediate and basal cells


5 A


1µm
5 B


6


0.5µm
7

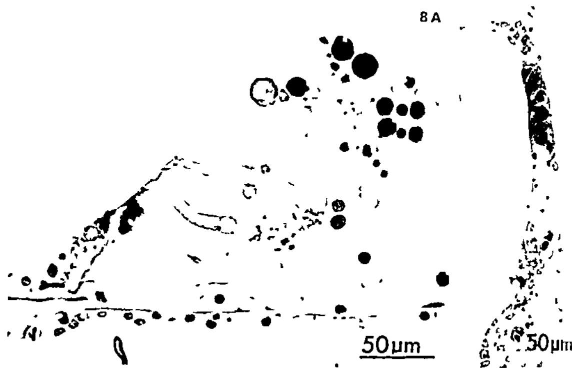


Fig 8 LM Combination of ethacrynic acid and atoxyl (A) Organ of Corti Oedema in the cytoplasm of the outer hair cell in the first row (B) Stria vascularis at the adjacent level Protrusion of strial cells into the endolymph

In specimens investigated shortly (1–12 hours) following the treatment, the organ of Corti appeared mainly unaffected If the animals were sacrificed 1–4 days later the outer hair cells were either severely degenerating (vesicular degeneration) or their place had at least in part been filled with proliferating supporting tissue The inner hair cells too were morphologically altered

A divergence from the normal position of Reissner s membrane could appear following the combination of ethacrynic acid and atoxyl During the first 12 hours after the administration of the drugs it often showed a hydrops labyrinthine After 1–2 days the membrane sometimes appeared depressed over the degenerating organ of Corti Specimens investigated 4 days following the treatment could show a normal position or a depression of Reissner's membrane However the hair cells had in these cases degenerated completely and were replaced by scar tissue

The effect of ethacrynic acid alone is mainly exerted on the basal coils of the cochlea in contrast to the atoxyl effect appearing primarily in the apical part of the inner ear Three guinea pigs were injected with 25 mg/kg of ethacrynic acid and 50 mg/kg of atoxyl The specimens were investigated after 24 hours and showed a normal ultrastructure of the stria vascularis in the two basal coils but in the third coil marginal cells protruded into the endolymphatic lumen (Fig 8*a–b*) The administration of separate but identical doses of ethacrynic acid and atoxyl does not affect the stria vascularis Multivesiculated osmiophilic bodies were sometimes found among the strial cells (Fig 7) in the late stage of ethacrynic acid induced change The organ of Corti appeared mostly normal in these cases though a mild oedema in some outer hair cells could occur

Administration of ethacrynic acid before atoxyl

Pretreatment with ethacrynic acid rendered the inner ear more vulnerable to the toxic

effect(s) of atoxyl A single dose of 50 mg/kg ethacrynic acid and 70 mg/kg of atoxyl did t damage the hair cells of the cochlea when ministered separately, whereas their com ed effects, although administered with an erval up to 12–18 hours, can cause irre rsible damage to the hair cells and an frequently occurred in the corre onding parts of the stria vascularis

ministration of ethacrynic acid er atoxyl

n injection of 35–50 mg/kg of ethacrynic acid hours following the administration of 70 g/kg of atoxyl did not cause morphological mages in the cochlea when the specimens ere investigated 4–7 hours following the last ection although Preyer's reflex was absent hen the investigation was made 1–3 days er when the reflex had returned, hair cells nd the stria vascularis were normal

DISCUSSION

The clinical reports by Pillay et al (1969) and Schwartz et al (1970) of ototoxic effects due to ethacrynic acid focused attention to the dangers that may arise following the use of potent diuretics which interfere with ion and water transport through plasma membranes The structural and physiological similarities between the stria vascularis of the inner ear and the kidney tubules are well established but hereby received additional confirmation

The results of the present investigation reveal the time course relationship of ethacrynic acid It has a rapid effect on the cochlea and may cause impairment or loss of hearing However these effects are—at least initially —reversible Ethacrynic acid induced ototoxicity has been reported by several investigators (Matz et al , 1969, Quick & Duvall, 1970 Ernstsson, 1972, Silverstein & Begin, 1974 Matz 1976) but whether or not this drug alone can produce permanent hearing loss in patients with normal renal function is still not settled An association between impaired renal function and the ototoxicity of this drug has been shown under controlled laboratory conditions in the guinea pig (McCrudy et al 1974) Ethacrynic acid induced damage is also reported with the concurrent administration of an aminoglycoside antibiotic (West et al 1973, Kaku et al , 1973)

Structural changes of the secretory epithelium may also be induced experimentally by means of atoxyl administration (Anniko & Wersäll, 1975 1977) However low dosages of this drug caused primary morphological damage to the hair cells at the apical part of the guinea pig inner ear (Anniko 1976*b*) Whereas the ethacrynic acid induced change of the stria vascularis is reversible in the early stage of toxic reaction the comparative changes found after atoxyl intoxication are mainly of irreversible type

The combination of ethacrynic acid and atoxyl has synergistic effects in their affection of cochlear hair cells which may be severely damaged while the corresponding parts of the stria vascularis appear morphologically less affected or even completely normal The administration of each substance separately in the same low dose did not however cause hair cell degeneration or persistent morphological changes of the stria vascularis The rejection of strial cells is probably an elimination process of irreversibly damaged biological material The intramitochondrial inclusion bodies may consist of accumulations of atoxyl but a degenerative transformation of the mitochondrial structure cannot be excluded Since many toxic substances act through a common morphological pathway of degeneration (Trumph & Ginn 1969) the appearance of intramitochondrial inclusions early during the cellular degeneration may indicate a primary site of toxic damage

Recent results by Bosher (1977) indicate that ethacrynic acid acts in three main phases Initially a rapid and short lasting (20–40 minutes) inhibition of active transport processes occurs followed by a delayed and long lasting (about

12 hours) mitochondrial dysfunction Also a reduction in the overall permeability of the endolymphatic system takes place, lasting about 12 hours However, tertiary effects arising from these alterations may furthermore cause an overall permeability change The ethacrynic acid induced change in the permeability of the endolymphatic partition is likely to increase the earlier known penetration of atoxyl into the cochlea (Anniko & Plantin, 1977) because of a probable dysfunction of the serum endolymph barrier of the inner ear (decreased threshold level for inflow to the cochlea?) Such a mechanism would explain the clinical findings of a rapid affection of hearing following ethacrynic acid administration during treatment with aminoglycoside antibiotics (Matz et al , 1969)

Both ethacrynic acid and atoxyl can affect the metabolism of endolymph as demonstrated by morphological changes in the stria vascularis Comparison of the electrophysiological response to ethacrynic acid and atoxyl (Cohn et al , 1974, Leonard et al , 1971, Bosher et al , 1973, Prazma et al , 1974) shows that both cause depression of the cochlear microphonics (CM) and the direct current potential (EP) Whereas the CM and the EP showed almost complete recovery within one hour of ethacrynic acid administration, the effects of arsacetin (acetylated atoxyl) persisted for a longer time Arnold et al (1976) reported that the EP decreased significantly when atoxyl induced morphological changes appeared in the stria vascularis and that the physiological and morphological alterations appeared almost simultaneously concerning the time course relationship

The effects of atoxyl at the cellular and subcellular level were analyzed by Thelestam et al (1977) reporting a direct damage to the plasma membrane (holes) but also an impairment of the protein metabolism occurred intracellularly

Kuijpers & Wilberts (1976) described how ethacrynic acid affects the ATP production intracellularly and concluded that this may explain the occurrence of a wide variety [illegible] changes that appear at various metabol[illegible] levels in the living cell due to ethacrynic acid

A combination of ethacrynic acid and atox[illegible] may impair or even totally block the end[illegible] lymph metabolism for so long time that th[illegible] ionic balance is altered and becomes toxic f[illegible] the hair cells In addition to the suggested i[illegible]crease in atoxyl penetration into the inner ea[illegible] an impairment of the atoxyl elimination fro[illegible] the endolymph via the stria vascularis m[illegible] also occur, thus exposing the cochlear ha[illegible] cells to toxic concentrations of atoxyl over [illegible] prolonged period of time

In clinical work it is of importance to co[illegible]sider the possibility of toxic interaction wh[illegible] combining two or several potentially ototra[illegible]matic drugs, even though they may have d[illegible]ferent site(s) and mode(s) of action at t[illegible] cellular/subcellular level and in doses whic[illegible] when administered separately, do not cau[illegible] ototoxic effects

ZUSAMMENFASSUNG

Nach Verabreichung einer Dose von Ethakrynsä[illegible] zeigen sich d e morphologischen Veranderungen in [illegible] Cochlea zuerst in der Stria vascularis der Basenspiral[illegible] als eine zunehmende intrazellulare Blasenbildung in d[illegible] [illegible] Öd[illegible] kann s[illegible] verble[illegible]

Die gleichzeitige Anwendung von Ethakrynsäure u[illegible] Atoxyl (einmalige Dosen) kann unwiederrufliche Schäd[illegible] an den cochleären Haarzellen und der Stria vascula[illegible] verursachen wahrend ihre separate Anwendung in [illegible] gleichen schwachen Dose keine Degeneration der Ha[illegible]zellen und ke ne bestehenden morphologischen V[illegible] [illegible] Ato[illegible] [illegible]

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Tabelle I *Zelldichte im Ganglion spirale (Zellen/0 01 mm²)*

	n	*M*	*s*
Normale Tiere			
Windung 1 (basal)	118	32 70	4 56
Windung 2	100	33 09	4 73
Windung 3	91	30 15	3 30
Windung 4 (apical)	29	31 01	3 56
Streptomycinbehandelte Tiere			
Windung 1 (basal)	260	30 99	7 11
Windung 2	201	29 56	6 04
Windung 3	253	27 15	5 28
Windung 4 (apical)	85	24 47	5 56

¹ Die Zelldichte wurde in n Zellen auf 0 01 mm² berechnet Die Mittelwerte der Zelldichten sind in der Tabelle mit M angeschrieben Die Unterschiede zwischen normalen und mit Streptomycin behandelten Tiere erwiesen sich als statistisch signifikant Die Abnahme der Ganglienzelldichte in unseren Fallen ist wesentlich geringer als die von Ostyn u Thyberghe n (1968) beschriebene die nach 30 tätiger Neomycingabe in der Basalwindung 25-75% betrug Die starkste Abnahme mit über 20% zeigte sich in der Spitzenwindung In den ubrigen Windungen lag die Abnahme zwischen 3 und 10%

DISKUSSION

Um eine Deutung der dargelegten Ergebnisse zu ermoglichen soll kurz auf bisher publizierte Wirkungen ototoxischer Antibiotika in der Cochlea eingegangen werden Im Bereich des Haarzell Lagers wird von den meisten Autoren (z B Kohonen 1965 Engstrom et al 1966 Spoendlin 1966 Stupp et al 1973 Wersall et al 1973) anfangs ein Zugrundegehen der außeren Haarzellen basal beginnend im weiteren dann ein Untergang der inneren Haarzellen apikal beginnend beschrieben Offensichtlich erfolgt anschließend eine Degeneration der zum Ganglion spirale führenden Nervenfasern (Kohonen 1965 Johnsson 1974) Da durch die Untersuchung von Spoendlin (1973) bekannt ist daß die uberwiegende Zahl der Ganglienzellen den inneren Haarzellen zugeordnet ist muß angenommen werden daß nach Zugrundegehen der inneren Haarzellen mit einer starkeren Degeneration von Ganglienzellen zu rechnen ist Dies wurde auch von Ylikoski et al (1974) nachgewiesen Der in unserer Versuchsreihe beobachtete Abfall der Ganglienzelldichte im apicalen Bereich konnte mit der Schädigung der inneren Haarzellen im apicalen Bereich zusammenhängen Die Ausbildung der sekundaren Degeneration erfordert einige Wochen da bei Fallberichten von Patienten mit Neomycin Intoxikation 17 Tage (Lowry 1973) bis 21 Tage (Lenhardt 1970) nach der Intoxikation postmortal noch keine Ganglienzellschaden bei vollig destruiertem Cortischen Organ nachweisbar waren Johnsson (1974) führt allerdings auch an daß der im allgemeinen bestehende Zusammenhang zwischen Haarzellschaden und Degeneration der Spiralganglienzellen in einer kleinen Zahl von Fallen nicht beobachtet wurde Es besteht also die Moglichkeit daß primär die Nervenfasern degenerieren ohne daß das Cortische Organ geschadigt ist In ähnlicher Weise wird von Bredberg (1973) ein Fall von symmetrischer Larmschwerhorigkeit beschrieben bei dem es zu einem beidseitigen Ausfall der außeren Haarzellen aber nur zu einer einseitigen Nervenfaserdegeneration in der Lamina spiralis ossea kam Fur die Erklarung der Degeneration von Spiralganglienzellen bei Einwirkung ototoxischer Antibiotika ware also auch eine direkte Schadigung der Nervenzellen zu diskutieren Die Untersuchungen von Stupp et al (1965) Voldrich (1965) und von anderen Arbeitsgruppen haben ergeben daß parenteral applizierte ototoxische Antibiotika in der Perilymphe einen weit hoheren Spiegel als in anderen Korperflussigkeiten erreichen Dieser hohe Spiegel bleibt sehr lange erhalten (Ilberg et al 1974) Der Zutritt der Perilymphe zu den Strukturen des Modiolus ist in mehreren Untersuchungen naher beschrieben worden (Arnold 1974 Wicke u Firbas 1974) und zeigt die Moglichkeit der Einwirkung toxi

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ZUR STREPTOMYCINSCHADIGUNG DES GANGLION SPIRALE

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Aus der I Hals Nasen Ohrenklinik und aus dem I Anatomischen Institut der Universitat Wien Osterreich

(Eingegangen am 1 Mai 1977)

Abstrakt Es wurde die Ganglienzelldichte im Ganglion spirale bei normalen und bei mit Streptomycin (3 Wochen 250 mg/kg taglich) behandelten Meerschweinchen unter sucht Es ergab sich eine signifikante Abnahme der Gang lienzellen mit dem Maximum der Abnahme in den Schnek kenspitzen Als Ursache dieser Abnahme werden sekun dare Degeneration nach Haarzellverlust oder direkte neurotoxische Schadigung der Ganglienzellen diskutiert

Die ototoxische Wirkung des Streptomycins ist seit seiner klinischen Erprobung im Jahr 1946 bekannt (Brown u Hinshaw, 1946, Glorig u Fowler, 1947, Hawkins et al , 1952) Wahrend immer wieder auch eine zentrale Lokalisation der Schadigung diskutiert wird (Winston et al , 1949), steht seit den Arbeiten von Stupp et al (1965) der periphere Angriffspunkt im Vordergrund Stupp et al konnten urch pharmakokinetische Untersuchungen eigen, daß parenteraler Applikation von Aminoglykosiden in Peri- und Endolymphe eine hohe, wesentlich langer als im ubrigen Organismus bestehen bleibende Konzentration der ototoxischen Antibiotika auftritt, die für die spezifische Organtoxizitat verantwortlich ist Als Substrat dieser Schadigung konnten morphologische Veranderungen an zahlreichen Strukturen der Cochlea beschrieben werden Dabei erregte das Schadigungsmuster der Haarzellen das meiste Interesse Uber Zellschadigungen im Ganglion spirale bestehen einzelne Berichte (Ruedi et al , 1953, Beck 1962), jedoch ohne genauere quantitative Angaben In den meisten morphologischen Arbeiten uber die Ototoxizitat wird auf das Ganglion spirale uberhaupt nicht eingegangen Lediglich bei Ostyn u Thyberghein (1968) und Ylikoski et al (1974) finden sich Prozentzahlen über die Zellabnahme im Ganglio spirale Es sollte deshalb das Ausmaß und di- Lokalisation von Nervenzelluntergangen u Ganglion spirale nach Streptomycineinwu kung untersucht werden

MATERIAL UND METHODE

Fünf junge Meerschweinchen mit einen Durchschnittsgewicht von 400 g bekamei durch 3 Wochen 250 mg/kg Körpergewich Streptomycinsulfat (Pfizer) pro Tag subcutar injiziert 6 Wochen spater wurden die Tiere dekapitiert, die Schlafenbeine isoliert unc diese nach Eroffnung der Bulla und des Vestibulum labyrinthi in 5% Formol fixiert Nacl Ylikoski et al (1974) ist nach 4 Wochen eine der Lasion entsprechende vollkommene Degeneration von Nervenzellen zu erwarten Di Entkalkung erfolgte in EDTA bei 5°C Nacl Einbettung in Paraffin wurden 15 nm dik ke Schnittserien angefertigt An Fotografier wurde in der früher beschriebenen Weise (Firbas et al , 1970) die Ganglienzelldichte be stimmt und mit der von Kontrolltieren vergli chen Die statistische Absicherung der Signi fikanz erfolgte mittels t-Test

ERGEBNIS

Die Schadigung des Cortischen Organs konnte an den Schnittserien nicht exakt beurteilt werden Es lag jedoch keine komplette Destruktion vor Die an den Schnittserien der verschiedenen Tiere erhobene Ganglienzell dichte ist in der Tabelle I zusammengestellt

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THE INFLUENCE OF COCHLEAR TEMPERATURE ON THE ELECTRICAL TRAVELLING WAVE PATTERN IN THE GUINEA PIG COCHLEA

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(Received July 23 1977)

Abstract Cochlear microphonics (CM) were recorded in the guinea pig using differential recording from the first and third cochlear turns as well as by using a 10-electrode array inserted in the scala tympani of the basal turn The excitation profile as represented by the CM was quantified by amplitude and phase data and was measured at normal temperature (38°C) and a 10° lower cochlear temperature It appears that the CM pattern shifts toward the base of the [illegible]

The electrical travelling waves recorded by intracochlear electrodes (Eldredge et al , 1971) are thought to reflect the movement pattern of the basilar membrane This cochlear micro potential (CM), which is variable in time is generated in the hair cells, and, as Dallos & Wang (1974) have shown, mainly in the outer hair cells In guinea pigs in which the outer hair cells were selectively destroyed by the application of kanamycin in ototoxic quantities the CM output was 30–40 dB less sensitive The CM generated in the outer hair cells is proportional to the displacement of the basilar membrane

A relatively large quantity of quantitative data concerning the movements of the basilar membrane has been published over the last few years Since von Békesy (1960) measured the movement and envelope of the travelling wave in cadaver ears, which required very high sound intensities (120–140 dB SPL), a number of more sensitive techniques have been used in this field With the Mossbauer technique, Johnstone & Boyle (1967) and Rhode (1971) obtained in vivo results at high frequencies and moderately high intensities (70–90 dB SPL) Wilson & Johnstone (1972) used a capacitive probe technique and Kohl loffel (1972) used laser illumination to measure basilar membrane movements All these investigations led to the determination of a high frequency slope for the movement of the basilar membrane of about 100 dB/octave and a low frequency slope of 10 dB/octave

It is assumed that the tuning of the basilar membrane depends to a large extent on the value of the compliance of the membrane which changes by about a factor 100 from base to apex (von Bekesy 1960) In addition there may be a small effect of viscosity loading due to the cochlear fluid but this seems to be restricted to the very low frequencies (Zwislocki, 1953) In model experiments von Bekesy (1960) observed that an increase in the viscosity of the fluid by a factor 10 caused a basal shift of about half an octave in the maximum of the travelling wave envelope Dahl & Kleinfeldt (1973) reported an increase in the viscosity of perilymph by a factor of 2 when the temperature was lowered from 38° to 28°C Cooling can therefore be expected to have an effect on the tuning of the basilar membrane, either by the small effect of viscosity loading of the basilar membrane or by the unknown effect on the compliance of the basilar membrane

scher Substanzen direkt auf den Nervus cochlearis Mootz et al (1972) beschreiben eine Schädigung der Gefässe des Plexus cochlearis nach Kanamycin Intoxikation Von Theopold (1976) wurde jedenfalls eine direkte neurotoxische Wirkung der Aminoglykosid-Antibiotika beschrieben Das Schadigungsmuster laßt in unserem Fall eher eine sekundare Degeneration nach Verlust von Haarzellen vermuten

SUMMARY

The neuronal density in the spiral ganglion was investigated in normal and in streptomycin intoxicated guinea pigs Streptomycin was administered in a dose of 250 mg/kg for 21 days 6 weeks after the streptomycin exposure a histological examination revealed a decrease in ganglion cell density in the spiral ganglion with maximum loss in the apical portion of the ganglion As mechanisms of the ototoxic effect a secondary degeneration of spiral ganglion cells after hair cell loss or a primary neurotoxic degeneration are discussed

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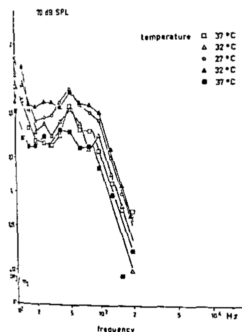


Fig 2 The ratio of CM amplitude from the first and third cochlear turns as a function of frequency at three cochlear temperatures The ratio is larger for lower temperatures and some hysteresis is present The shift of the curves is uniform

Laboratories type DL 102S) and an XY recorder (Hewlett Packard, type 7035B) permitting a time resolution of 0 02 msec in the recordings

In experiment 2, the CM was recorded from the scala tympani electrodes with reference the cervical muscles The recorded CM was amplified 10 times, each electrode having own pre amplifier All pre-amplifiers are identical and have flat response curves from 8 Hz 10 kHz The pre amplifier of the basal electrode (no 0) is permanently connected to a main amplifier (500–25 000 times), those of electrodes 1 to 10 are successively connected to the second main amplifier With a gain-phase meter the amplitudes and phase differences between electrodes 1 to 10 with respect to electrode 0 are recorded

Temperature control

The animal s temperature was measured both rectally and in the oropharynx with NTC resistors The cochlear temperature was assumed to be almost the same as that of the oropharynx, but it is possible that cooling and warming of the animal leads to some lag in the cochlear temperature

Therefore, the compound action potential latency (AP) for a high frequency tone burst was recorded before and after each series of measurements and during the cooling and warming periods Since it is known that the AP latency is very sensitive to temperature as well as to other metabolic disturbances (Gannon et al , 1966, Eggermont 1974) we made it a condition that the beginning and end latencies for a complete experiment be identical Coats (1965) reported an increase in AP latency with decreasing temperature amounting to 0 06 msec/°C, while Eggermont (1974) reported a change amounting to a factor of 1 6 per 10 C temperature

RESULTS

Experiment I Differential Electrode Recording

Cochlear microphonic amplitude

The CM amplitude measured in μV RMS was determined for 9 guinea pigs in the first and third cochlear turns at two temperatures normal (37°C) and 10°C lower The mean values are plotted in Fig 1 The output of the first cochlear turn is relatively constant over the whole intensity range, whereas the third turn responds only to frequencies below 2 kHz When the cochlear temperature is lowered the output of both turns decreases

A convenient way to demonstrate a differential effect of cooling on the output of both cochlear turns is determination of the amplitude ratio CM_3/CM_1 This is illustrated in Fig 2 for one animal The temperature sequence comprises 5°C steps in the following order 37°, 32°, 27°, 32° and back to normal It is clear that the CM_3/CM_1 ratio increases when the temperature is lowered and that this trend continues in the initial part of the rewarming period The initial and final series are, however, almost

Under the assumption that the CM provides a representation of the tuning properties of the basilar membrane (Dallos, 1973a), we studied the influence of temperature on the electrical travelling waves in relation to possible changes in the mechanical tuning of the cochlea

METHODS

Surgical procedure and electrode placement

The data reported here are based on experiments performed in 13 guinea pigs. Premedication consisted of 15 μg atropine sulphate per kg body weight, 5 mg promethazine hydrochloride, and 5 mg chlorpromazine hydrochloride applied intramuscularly 30 minutes preoperatively. A moderately deep anaesthesia was obtained with urethane administered intraperitoneally at a dose of 1 g/kg body weight. Tracheotomy was routinely performed, and the cochlear bulla of the right ear was exposed via the standard latero-ventral approach described by Tasaki & Fernandez (1952).

In experiment 1, nichromesteel wire electrodes with a diameter of 25 μm were introduced into the scala tympani and scala vestibuli of the first and third cochlear turns, one pair 4 mm and the other pair 14 mm from the stapes. In experiment 2, a multi-electrode array consisting of 11 electrodes about 0.2 mm apart, was inserted into a slit in the scala tympani of the basal turn according to Kohllöffel (1971). The animal was cooled according to the procedure described by Eggermont (1974) and placed in a sound-treated room which was shielded electrically

Stimulation, calibration, and recording

Continuous tones and tone bursts were used as stimuli and presented via a STC-4026A dynamic headphone attached to a modified ear speculum, giving a closed sound system of about 5 cm³. With a calibrated probe microphone (Bruel & Kjær cathode follower type 2615) and a ½ inch cartridge (type 4133) in combination with a probe 1 6 mm in diameter and 15 cm long the sound pressure level, at a point a

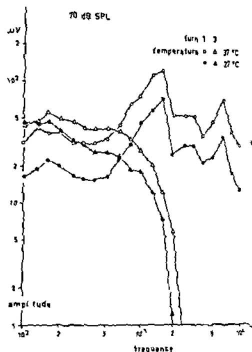


Fig 1 The amplitude of the CM as a function of quency for two cochlear temperatures at 70 dB S Lowering of the temperature results in a decrease in amplitude

few millimetres from the eardrum, was reco ed during the experiments The continu tones were generated by a Rhode & Schw (type FTA) wave analyser with a coupled generator and passed to the headphone vi calibrated attenuator (1 dB steps) The bursts always started at a zero-crossing of sine wave (for details, see Spoor, 1974)

In experiment 1, the responses recor from the scala vestibuli and scala tymp were pre-amplified 10 times and then fed a differential amplifier. A compensation work (Tasaki et al , 1952) was used to separ the AP and SP from the CM. The CM out was measured after a total amplification of to 25000 times, using the wave analyser wi Hz band width. Phase measurements w performed, without filtering, with a gain-ph meter (Hewlett-Packard, type 3575A) For measurements of travelling wave delay, CM response to low-frequency tone bur was recorded with the use of an averager (D

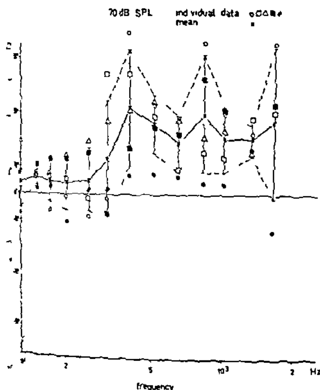


Fig 4 The increment in phase difference between the first and third cochlear turns due to cooling as a function of frequency In the middle frequencies an average increase of 23 degrees difference is observed after lowering the temperature by 10°C

of 35 5 and 28°C Consistent results were obtained in 4 animals

A representative case is shown in Fig 5 (*a*, *b*, *c*) for various stimulus frequencies

The phase data for the lowest frequency used (1 kHz) can serve as control, since at this frequency the wavelength of the travelling wave will be about 10 mm, which is large with respect to the width of the electrode array (2 mm) Consequently, little phase variation is to be expected and the amplitude distribution should be uniform due to the lack of phase cancellation

With increasing frequency, the CM amplitude decreases for all electrodes in the array and progressively toward the more apical electrodes. The phase gradient across the electrode array increases progressively with stimulus frequency leading to phase reversal (e g at 13 kHz the phase difference exceeds 180°)

Cooling causes no systematic changes in the phase difference at low frequencies For higher frequencies the phase difference increases, which is in accordance with a basal shift of the travelling wave profile From Fig 6 it is quite clear that around 13 kHz for all electrodes there is a shift of the phase reversal of the order of 250 Hz due to the cooling As Fig 5 (*a* *b*, *c*) shows, the CM amplitude decreases as a result of cooling and in conformity with the results of experiment 1 in the basal turn For high frequencies, i e 12 kHz and higher the CM amplitude increases slightly for the more apical electrodes At 13 kHz cooling causes the phase reversal to shift from electrodes 9 and 10 to electrodes 7 and 8 The corresponding amplitude minimum is situated at the same electrodes These results point to a basal shift of the CM pattern with one or two electrode distances (i e 150–300 μm)

DISCUSSION

In experiment 1, the CM profile was studied with the use of amplitude as well as phase data Phase differences reflect travelling wave

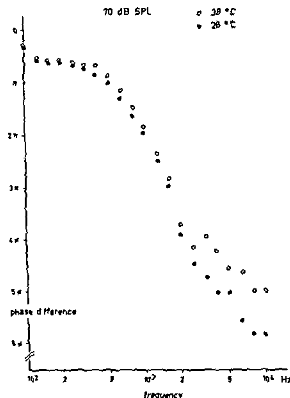


Fig 3 Average phase difference between the CM recorded from the first and third cochlear turns A frequency-dependent phase difference increasing from an almost constant value of $\pi/4$ radians at low frequencies toward 4π radians at 2 kHz is evident As a result of cooling by 10°C, the phase difference increases by about $\pi/8$ radians at a given frequency This is thought to be caused by a shorter travelling wave length at lower temperature for the frequencies in this part of the cochlea

the same For the whole series of 9 animals, the average shift in frequency producing the same CM_3/CM_1 output at both temperatures was calculated This was done for CM_3/CM_1 values of 1 2, 1 0, 0 5, and 0 2 It was found that the frequency shift was not systematically dependent on the segment of the curves under consideration, indicating a uniform shift of the curves as a whole The value of this shift, which is 1 3±0 2, points to an upward shift in frequency amounting to about 1/3 octave

Cochlear microphonic phase

Between the CM differentially recorded from the first and third cochlear turns there is a phase difference For the group of guinea pigs under study, the average phase difference b[e]tween these two turns is plotted as a functi[on] of stimulus frequency at two different te[m]peratures in Fig 3 At low stimulus frequenc[ies] the average phase difference amounts to $\pi/$[…] $\pi/2$ radians, increasing up to 4π radians a[t] kHz Values above 2 kHz are probably due [to] remote contributions, and must be consider[ed] with reserve

Lowering of the temperature by about 1[0°] results in an increase of the phase differe[nce] at a given frequency amounting to about π[…] radians This is visualized in Fig 4, wh[ich] shows the individual phase differences in ea[ch] of the 9 animals together with the mean va[lue] and standard deviation (area between dash[ed] lines) It is evident that in general there is [an] increase in phase difference which amount[s to] about 5° up to 300 Hz and then rapidly [in]creases and stays at a value of about 20° up [to] 2 kHz Values above 2 kHz have been omit[ted] For the region above 500 Hz, there was an [in]crease in phase in all but one case

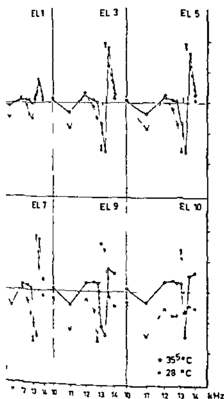
Electrical travelling wave delay

When stimulation is performed with short to[ne] bursts, the CM recorded from the first a[nd] third cochlear turns shows a characteristic [lag] in onset, amounting to between 0 65 and [...] msec, depending on the interelectrode [dis]tance A series of measurements was [per]formed with tone bursts of various frequen[cies] below 1 kHz at the normal temperature and [10°] below the normal temperature

For 6 guinea pigs the average lag was 0,[…] 0 1 msec at 37°C For the 10° lower temp[era]ture the lag increased slightly and amounte[d to] 0 9±0 1 msec, giving an average shift of [...] msec ($p<0\ 005$)

Experiment 2 Multi electrode Array Recording

The amplitude and phase distribution of [the] CM is measured along the electrode array [for] stimulus frequencies ranging from 1–14 kH[z at] a level of 90 dB SPL for cochlear temperat[ure]



The CM phase as a function of frequency at dif temperatures Cooling causes an increase in phase ment in accordance with a basal shift of around lz

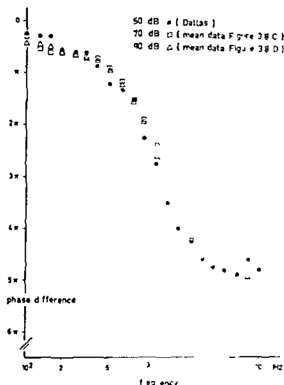
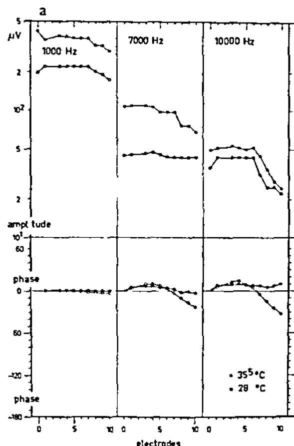
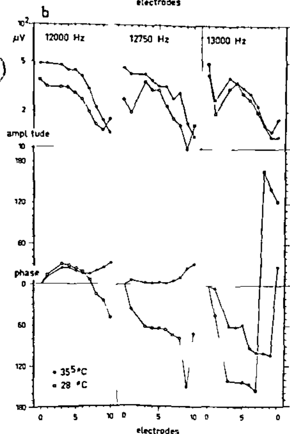


Fig 7 The phase difference b CM recorded from the first and the third cochlea The frequency dependent accumulating phase obtained in experiments at normal body temperature in agreement with the phase difference calculated experiments by Dallos (1973*b*)

phase difference between the first and cochlear turns and compared the result our experimental data (Fig 7), which ed marked agreement On the basis of the se shift Dallos calculated the travel time of CM from the first to the third cochlear s to be about 1 1 msec In our direct measents a value of 0 8 msec was obtained increased travel time for the lower temture corresponds to a larger phase differe than was measured directly A larger se difference for a given stimulus frequencorresponds to a basal shift of the maximum the travelling wave envelope The problem s why the amplitude data and the phase a show opposite effects Since, as already d the metabolism of the hair cells is inenced by the cochlear temperature, the temture coefficient may have been different for the first and third cochlear turns This is illustrated in Fig 8 where the temperature coefficient for the CM recorded in both turns at 70 and 90 dB SPL is plotted (averaged data for 9 guinea pigs) Here the temperature coefficient is about 0 5 for the first turn and about 0 9 for the third turn at least for sufficiently low frequencies The temperature coefficient in the third cochlear turn appears to be frequency dependent which points either to remote contributions from the higher turns since the temperature coefficient drops to the first turn value or else to a frequency dependent non linearity that is also temperature dependent

These problems did not arise in experiment 2, where all the recordings are from the basal turn and show a conformity between amplitude and phase data in that a cooling of about





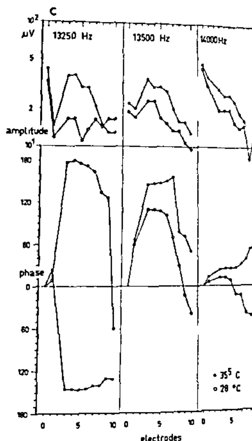


Fig 5 (a–c) The CM amplitude (in µV RMS) and the phase grad ent along the electrode array at various quencies and different temperatures at 90 dB SPL C ing leads to an increase in phase difference and a b shift of the phase reversal along the electrode ar This is in accordance with a shift of the CM excit pattern of about 250 µm

delays and tuning characteristics of the bas membrane while amplitude data also bear the state of the CM generators i e the m bolism of the hair cells From the amplit data it may be concluded that the amplit profile shifts toward the apex when the t perature is lowered However the phase c and travelling wave delays are consistent w a shift toward the base of the cochlea T there seems to be a contradiction

Dallos (1973*b*) measured the phase at between the differentially recorded CM for first, second and third cochlear turns and sound at the eardrum for an intensity of 50 SPL From his data we calculated the cum

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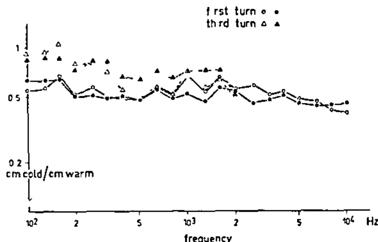


Fig 8 The average ratio of the CM corded at low and normal function of frequency For the first the ratio is frequency independent amounts to 0 5 For the third turn the is frequency dependent and is about 0 low frequencies which indicates a d effect of temperature in the first and turns

8°C produces a basal shift of the excitation pattern along the cochlear partition This basal shift means that the compliance of the basilar membrane increased when the temperature was lowered Although this may sound a bit unphysical at first, because one expects cooling to make the basilar membrane stiffer, the results agree with those obtained by Eatock & Manley (1976), whose recordings from single auditory nerve fibres in the gecko showed that the characteristic frequency of the fibre increased when the temperature was raised This indicates an apical shift of the stimulation profile under an increase in temperature

The increase in viscosity of the perilymph (Dahl & Kleinfeldt, 1973) will not have any influence on the phase data according to Zwislocki s (1953) theory of cochlear mechanics, but may influence the amplitude distribution, especially in the apex of the cochlea The effect will be a slight basal shift of the travelling wave envelope maximum

The smallness of the effects on the mechanical tuning produced by cooling of the cochlea suggests that some counteracting effects might be responsible for these results On the basis of two independent sets of measurements it remains difficult, however, to consider other mechanisms besides the increased compliance and increased viscosity responsible for the effects observed

ZUSAMMENFASSUNG

Mikrophonpotentiale wurden von der ersten und dri ... chens abgele

tur registriert worden Es zeigt sich daß die ... der Erregung einer bestimmten Reizfrequenz in Richt ... Schnecke

folge der Kuhlung der Schnecke

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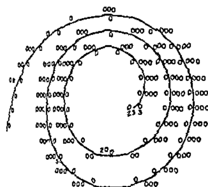
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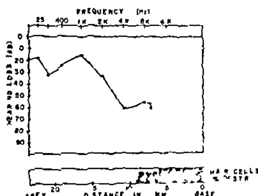
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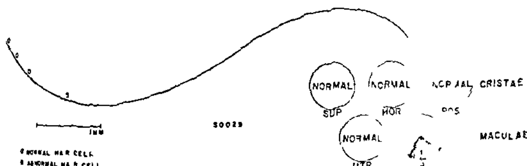
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There was damage to the ... of the macula of the saccule which was e t m

...e ultrasound literature provides more describing clinical findings than data re-...ing the effect of ultrasound upon the audi- and vestibular systems Long term fol-...up studies do appear to indicate improve-...t with ultrasound (Stahle, 1976) How-...r there must be crucial differences, for as ...(1973) pointed out, the results of ultra-...ic therapy vary considerably among sur-...ns Certain data is available regarding the ...ng effects of ultrasound on sensory ele ...ts supporting structures, and endolym-...atic systems (Portmann et al , 1952, Brain ...al 1960 McLay et al , 1961, Formby, 1963, ...et al , 1971, Crysdale & Stahle, ...7)

Little behavioral experimental data has been published regarding the round window ...rasound approach McGee et al (1963) have ...rted on the effect of direct stimulation of the cochlea by a relatively high dose of ultra-sound However little experimental data is available on the effects of the currently used levels of ultrasound upon the auditory function using the round window technique

The purpose of the present study was to investigate the effect of ultrasound irradiation presented through the round window with emphasis upon auditory effects of treatment Sound-conditioned cats were used to allow evaluation of functional changes in the auditory system The levels of ultrasonic irradiation approximated those levels at present being used with the round window approach (Basek, 1970, Kossoff 1972)

METHODS AND PROCEDURES

Thirteen healthy adult animals were used in the experiment These comprised 2 sham audiometric control animals, 2 sham histological control animals and 9 experimental ani-

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ULTRASONIC IRRADIATION THROUGH THE ROUND WINDOW

Functional and Morphological Findings in Sound conditioned Cats

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(Received June 15 1977)

Abstract Ultrasound was presented through the round window in 9 sound-conditioned cats at levels approximating clinical usage Threshold shifts were mild to moderate and confined to 4 kHz and higher There was total loss of function at 16 kHz in 2 animals Threshold shifts correlated with cochlear histological findings as shown by reconstruction There were three main types of lesions abnormalities of supporting structures hair cell loss and lesion of Reissner s membrane Supporting structure damage was the most frequent The cristae of the semi circular canals did not show any lesions though some saccular damage was noted These changes were not observed in 4 control animals Some conductive damage was noted as a result of probe placement High frequency loss can be expected with the round window approach at irradiation levels of 52 mW for 20 min or stronger

The use of ultrasound has become increasingly widespread as a treatment for Meniere's Disease Ironside & Lindsay (1959) have pointed t that ultrasound had been used for the treatent of otosclerosis as early as 1928 Reports of treatment for various ear disorders had also appeared in 1948, though with considerably varying results (Arslan, 1953) The use of ultrasound has been pursued more extensively since Krejci described in 1949 applying it to the vestibule (Aragno, 1960)

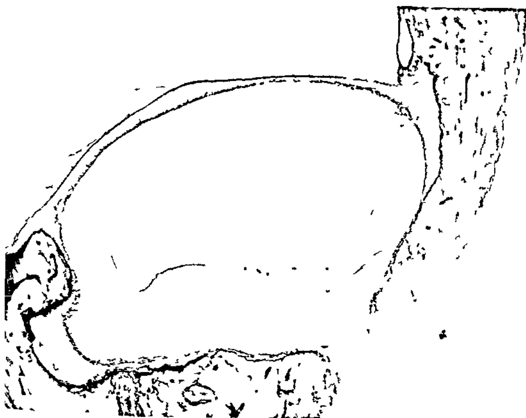
The purpose of using ultrasound, for Meniere's symptomatic treatment, was to eliminate the vestibular part of the labyrinth while attempting to maintain hearing function The semicircular canal approach had been used almost exlusively for clinical treatment until the mid sixties Much of the development and clinical experience can be ascribed to Arslan (1953), Krejci (Aragno 1960), James (1965, James et al 1960) among others Kossoff & Khan (1966) suggested that an improved technique would in itself be advantageous A method of directing the ultrasonic beam directly through the round window was presented as that alternative (Kossoff et al 1967)

It was felt that the round window approac eliminated several disadvantages of the sem circular canal approach Some of these we unnecessary mastoidectomy, potential risk hearing loss, possible facial nerve damage an inefficient transmission of ultrasonic wave The round window approach provides a mor direct (primarily fluid) pathway for the ultr sound irradiation This is important since th absorption coefficient is much higher for bon than it is for fluid In approaches requirin transmission of the ultrasonic waves throug bone (e g semicircular canal approach) co siderable energy is absorbed by the bone an dissipated in the form of heat In early expe ments this heat itself became a problem du ing ultrasonic presentation

Since the introduction of the procedur using the round window, several investigato have presented clinical findings Some co pare the round window vs other approache (Arslan, 1968, Basek, 1973) Others provid evidence that tends to establish the adv tages of the round window approach (Kosso et al , 1968, Basek, 1970, Kossoff, 1972)

Study conducted in part Otological Research Laboratorie Wayne State University School of Medicine Partiall supported by a grant from the Deafness Research Found tion

Findings were reported in part at the XV Pan America Congress of Oto-Rhino-Laryngology and Bronch Esophagology in New Orleans Louisiana Novem 1976



3 Photomicrograph of the round window which s thickening of the *round* window membrane with exudate partially blocking the niche in the animal described in Fig 1 Hematoxylin-eosin ×52

s Eleven animals underwent preliminary culectomy (Paparella & Hohmann, 1962) labyrinthectomy followed by audiometric nd-conditioning and threshold determina- (Culler et al, 1935, Schuknecht, 1953, ott et al, 1960) The t o histological con- s underwent shold Their fter

tracheotomy was performed and a small tube was attached with silk sutures to allow adequate ventilation A bulla approach was performed and adequate exposure obtained with a selfretaining retractor The mucosa was elevated from the bulla and a small shell of bone was removed by drilling around close to the margins of the bulla

The bulla was filled with distilled water The ultrasound probe was placed in the round window niche and held by a mechanical support Bleeding was minimal, if blood did block round window, it was gently aspirated and lled water again applied

ultrasonic irradiation was presented via ultrasonic stimulator using a 1 1 mm transducer in a 1 5 mm diameter probe A similar sized sham probe for all control animals The apparatus previously described by Kos

A

B

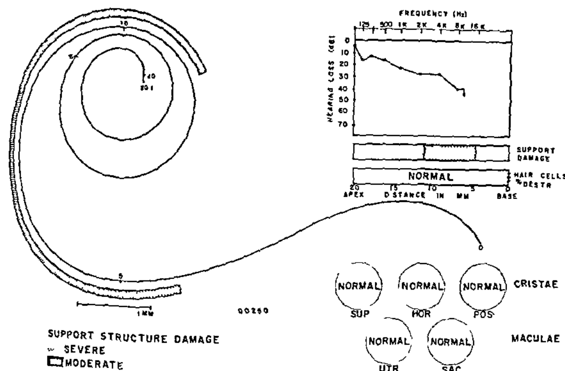


Fig 5 Cochlear reconstruction of a medium level animal showing supporting structure damage from 4 25 mm to 1 mm along the cochlea This corresponds to frequency hearing losses seen from 2 kHz to 16 kHz The hair cell population is normal No abnormalities are seen in the vestibular labyrinth

off et al (1967) It was not necessary to irrigate the bulla during irradiation Kossoff (1972) has established that with this apparatus and the low level of ultrasound irradiation used, temperature rise was not sufficient to be destructive The levels of stimulation and irradiation times were low (approximately 1 mW/10 min), medium (approximately 54 W/20 min), and high (approximately 110 W/20 min) Since monitoring of the output level is not possible, it is essential to perform calibration before and after application A method of calibration described in the irradiator manual was found to be satisfactory The output is measured by observing weight changes with a sensitive analytical balance The levels determined pre- and post-operatively were usually quite close (±0 5 mW) However, actual output did not correspond to the manual settings on the equipment

At the end of the appropriate stimulation, the distilled water was gently aspirated and probe removed The shell of bone was replaced and sutured with wire The skin and subcutaneous tissues were approximated and sutured Tracheostomy tube was removed Clinical observations were made post-operatively, as were repeated threshold determinations, approximately 2 weeks, one month, and 2 months post-operatively. Intravital perfusion was performed and temporal bones removed and prepared for processing (*Schuknecht, 1953*).

Fig 4 (A) Photomicrograph of the macula of the saccule showing moderate hair cell loss in the sensory epithelium


A
B
B



Fig 7 Photomicrograph of the posterior crista showing normal sensory epithelium in the same medium level animal reconstructed in Fig 5 Hematoxylin-eosin ×240

FINDINGS

unctional findings

here were minimal clinically observable estibular alterations after ultrasound irradiation In 4 animals, there was slight nystagmus towards the operated ear for 2–3 days postoperatively However, there were no symptoms that would suggest labyrinthectomy

Audiometric functional findings can be summarized as follows

A Animals that showed no significant change (within 10 dB of threshold) This group included the audiometric control animals and 2 other cats, one that had received low irradiation and one that had received medium A slight improvement was noted between the first post-operative audiometric testing (?

ig 6 (A) Photomicrograph of a normal organ of Corti n the animal shown in Fig 5 at 14 5 mm along the cochlea The supporting structures can be seen firmly attached to the basilar membrane (B) Photomicrograph of the organ of Corti at the 8 mm region of the cochlea in the same animal The supporting structures are pulled away from the basilar membrane leaving the Böttcher's cells (B) The hair cells are normal Hematoxylin-eosin ×450

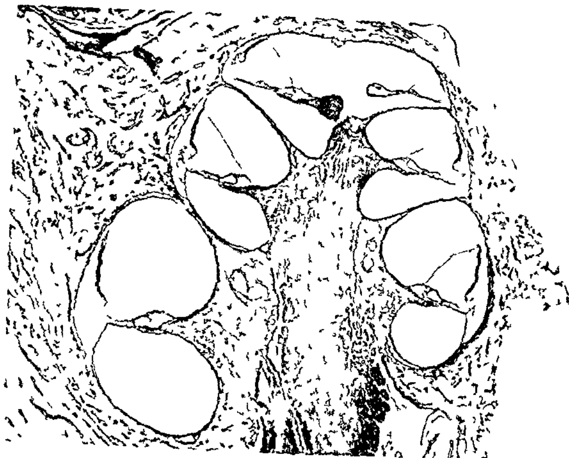


Fig 8 Photomicrograph of the cochlea from a high level irradiated animal The Reissner s membrane is missing in the basal turn only Hematoxylin-eos n ×32

eeks) and the final post operative testing (2 months) in the control animals

B Two animals showed moderate threshold shifts throughout post operative testing which was greatest for the lower frequencies Both were of the low irradiation group One of them had a quite marked progressively deteriorat ing threshold at all tested frequencies This animal was found to have otitis media

C Two animals showed slight to moderate high frequency shifts (one medium and one high level) In these cats the high frequency shift stayed rather stable throughout the post operative testing In one there was an im provement in the lower frequencies similar to that noted in the control animals

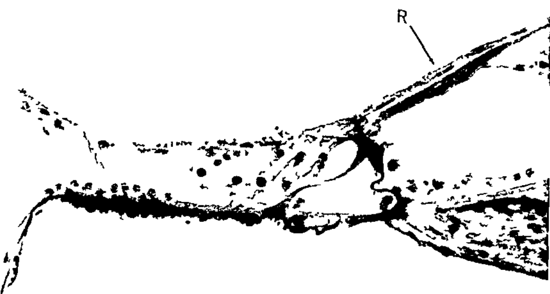
D Three animals showed moderate to se vere shift of threshold values in high fre quencies (2 medium and one high level) Two exhibited total loss at 16 kHz Two ani showed some shift in the threshold value the lower frequencies as well In one cat (dium level) the threshold further deteriora after the first post operative session m marked at the high frequencies However other 2 animals showed rather stable high quency losses

Thus the audiometric findings indicated the medium to high level animals a high quency shift more noticeable from 4 kHz t

Fig 9 Photomicrograph showing the comparison o normal position and collapsed Reissner s membrane cochlea of the an mal shown in Fig 8 (A) Normal pos of Reissner s membrane at the 15 mm region (*R*) The lapse of the tectorial membrane is an artifact seen thro out the cochlea (B) Reissner's membrane (*R*) is above the tectorial membrane extending a ross the hair cells in the 8 mm region Hematoxyl n-eosin ×

R

A



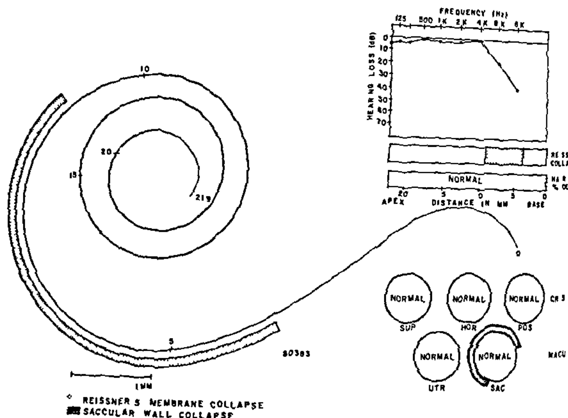


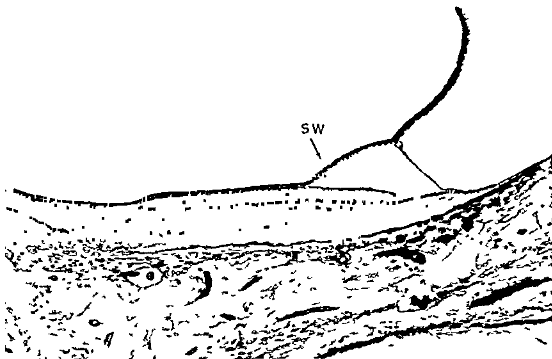
Fig 10 Cochlear reconstruction in the high level animal *shown in Figs 8 and 9* The rupture and collapse of Reissner s membrane extends from 4 mm along the cochlea to 9 3 mm This corresponds to the hearing loss at 8 kHz and 16 kHz The saccular wall also shows collaps tending over 60% of the macular surface The hair were normal as was the rest of the vestibular labyrin

kHz All losses for the low irradiation animals appeared to be conductive in nature There were some conductive shifts in the medium and high animals as well These conductive changes had a tendency to improve, as seen in the control animals There was a high frequency loss due to the ultrasonic irradiation However, the medium level and the high level dosages used in the present experiment showed no difference based on functional findings

Histopathological findings

A survey of the histological findings of the ultrasound irradiated animals showed three basic types of damage These were actual hair cell loss, abnormalities of supporting structures of the organ of Corti, and lesion of Reissner s membrane Representative animals from each group will be discussed along with sociated vestibular histology

A *Hair cell loss* Fig 1 shows a coch reconstruction and audiogram for a med animal (52 2 mW/20 min) that showed ac hair cell loss There is a lesion of the orga Corti consisting of loss of outer hair cells ginning at the lower basal turn and exten towards the hook of cochlea where the loss cludes inner hair cells The hair cell loss responds to the high frequency threshold s shown in the audiogram Neuron populat in the spiral ganglion was normal through There is a loss in the lower frequencies well, *which may be a result* of blockage of round window There was also moderate l (about 20%) of hair cells in the macula of saccule Fig 2 makes a comparison betw supporting structures at about 8 mm regi



11 Photomicrograph of the saccular wall (sw) over macula of the saccule in the cat shown in Fig 10 ıatoxylin eosin ×120

cochlea with a more apical turn at the mm region In 2B, the loss of outer hair ls can be seen, whereas in 2A, all hair cells present The outer hair cells were de ɔyed more apically than were the inner hair ls which only showed damage, extremely ıally and toward the hook Fig 3 shows : round window in this animal The mem ıne is severely thickened, with the round ndow niche showing fine granular exudate rtially blocking the window Fig 4 illu- ates the damage to the macule of the sac le There is moderate hair cell loss in the nsory epithelium (4A), with rupture of the ccular wall inferiorly (4B)

B *Abnormalities of supporting structures* ig 5 is the cochlear reconstruction of another edium level animal (53 mW/20 min) There as no hair cell loss throughout However, ere was detachment of supporting structures om the spiral ligament and from the basilar ıembrane The area of damage corresponds closely to shifts noted in the final post operative thresholds The most prominent loss occurred at 8 kHz and 16 kHz It is interesting to note that the shift at 2 kHz and 4 kHz was not apparent until one month after irradiation The mild losses from 62 5 to 2 kHz correspond to moderate severe thickening of the round window with serofibrinous fluid in the niche Findings were normal throughout the vestibular portion of the inner ear Fig 6 makes comparison between the supporting structures at the 8 mm region of the cochlea (Fig 6B) with a more apical turn at the 14 5 mm region (Fig 6A) In the basal portion, Bottcher's cells can be clearly seen attached to the basilar membrane, while the supporting structures have become detached Fig 7 shows normal sensory epithelium of the crista of the posterior semicircular canal

C *Lesion of Reissner's Membrane* Fig 8 shows absence of Reissner's membrane in the basal turn This was noted in a

dosage animal (110 mW/20 min) As can be seen in Fig 9, the membrane appears to have fallen across the tectorial membrane at the 8 mm region of the cochlea (Fig 9B) in contrast to the appearance in the 15 mm region (Fig 9A) which is normal The collapse of the tectorial membrane is an artifact seen throughout the cochlea Cochlear reconstruction and final thresholds are presented in Fig 10 The mild to moderate shift at 8 kHz and 16 kHz corresponds to the area of rupture and collapse of Reissner's membrane In this animal, there was mild reaction at the level of the round window This corresponds to a near-absence of shift in thresholds 4 kHz and below There was, in addition, a rupture of the saccular wall with collapse over 60% of the macular surface The sensory elements remained intact The collapse is illustrated in Fig 11

The findings, apparently the result of ultrasonic irradiation, have corresponded closely to high frequency threshold losses Other audiometric changes, 2 kHz and below, have consistently been correlated with changes of a conductive nature Most common was the thickening and blockage of the round window membrane This was noted to a varying extent in all animals, both sham and experimental Damage within the vestibular system was minimal, usually confined to the saccule In the 5 animals showing threshold shifts, 3 cases were a result of supporting structure damage, one of hair cell loss, and one of collapse of Reissner's membrane

DISCUSSION

Portions of the present experiment are comparable to other investigations Basek (1970) was able to show, histologically cochlear damage in the basal turn (hair cell loss), some saccular damage, and damage to the posterior cristae Basek used an irradiation level of 30 mW/10 min, which was chosen as the lowest irradiation level in the present investigation The low level animals in this study did not exhibit such damage However, he find a considerable variability ranging f minimal damage to actual hair cell loss In present investigation, similar damage noted when the irradiation level was incre to 20 min at either 52 mW or 110 mW though the area of damage was in the b turn of the cochlea, only one animal show significant hair cell lesion Most of the cc lear damage was due to abnormality the supporting structure The changes served in the vestibular system were confi to the saccule The three cristae of the s circular canals remained remarkably intact

Although the point of ultrasonic applica was different, the behavioral functional t ing was similar to that of McGee et al (1 By applying ultrasound irradiation direct to basal turn of the cochlea they caused a marily high frequency loss that impro gradually during the course of testing rem ing poorest at the higher frequencies The improvement seen in the present investiga was in the lower frequencies It is felt that might have been due to observed chan around the round window area and would be present in the surgical approach us the McGee study Once a high frequency l did appear at the first post-operative tes at 2 weeks, it remained quite stable This frequency loss did show considerable va tion, ranging from no change to complete l of function at 16 kHz Kossoff (1972) u the round window approach and similar radiation levels showed no shift in cochl microphonics performed immediately afte radiation

Although hair cell lesion was found in c one animal, it is a very common finding w ultrasonic irradiation It is much more not able with more intense stimulation or stim tion closer to the cochlea (Portmann et 1952, Brain et al , 1960 Formby, 1963 Bas 1970) In addition, further agreement noted with McGee et al (1963) and Crysdal Stahle (1972) in that the outer hair cells see more severely damaged

The most frequently observed damage, histologically, that was correlated with significant functional shifts, was abnormality of the supporting structure Portmann (1952) had described damage of support structures during ultrasonic stimulation, a method which applied ultrasound through the external auditory meatus Paparella & Melnick (1967) have described a similar uplifting from the basilar membrane of the support structures into the endolymph This was caused by direct stimulation of the ossicles with a high speed drill It is felt that in most previous investigations, higher levels of ultrasound were used, thus causing more severe damage than the supporting structure damage found in the present study A similar type of separation has been observed by Lundquist et al (1971) in the cristae, where sensory cells and supporting structures showed separation from the underlying stroma in the cristae

The other finding, rupture and collapse of Reissner's membrane, was similar to the finding of McLay et al (1961) in the guinea pig However, audiometric changes were not determine in that study A threshold shift solely as a result of collapse of Reissner's membrane has not previously been documented The associated collapse of the saccular wall was also quite common, especially where there was a total collapse of the membranous labyrinth due to high level ultrasound irradiation or irradiation directly to the semicircular canal (McLay et al , 1961, Formby, 1963, McGee et al 1963, Basek, 1970) Arslan (1968) has pointed out that as a result of the angle of the niche of the round window, more damage would be expected in the basal turn of the cochlea and in the saccule

In the present investigation, there was no evidence of a deficit in the sensory epithelium of the cristae Crysdale & Stahle (1972) have noted the rarity of complete vestibular destruction with the semicircular canal approach However, successful use of ultrasound using the round window approach has been reported for treatment of Meniere's disease (Kossoff et al , 1967, Basek, 1970, Base 1973) The procedure apparently does not i volve total destruction, especially with t round window approach and the current used levels Some moderate hearing loss c be expected at higher frequencies, with pc sible total loss at 16 kHz Losses also may caused by the placement of the probe in t round window niche, especially where drilli may be performed for better probe irradiati angle

ACKNOWLEDGEMENTS

We are indebted to Mr Yong Ku Choe for histologi preparation and to Mr Arthur Bowden for photomic graphy

ZUSAMMENFASSUNG

Ultraschallbehandlung ahnlich derer haufig in klinisc Anwendung wurde durch das runde Fenster an ne schallkonditionierten Katzen vorgenommen Horschw lenveranderungen waren leicht bis maßig und beschra ten sich auf Frequenzen 4 KHz und höher Zwei Ti hatten totalen Funktionsverlust auf 16 KHz Horschw lenveranderungen bezogen sich auf cochleare hi logische Befunde Die Korrelation wurde durch Rek struktion bewiesen Drei typische Hauptladierungen w den beobachtet Abnormalitaten der Stutzstruktur Verlust von Sinneszellen und Ladierung von Reissn Membrane Schadigung der Stutzstruktur wurde haufigsten bemerkt Die Kristae der halbkreisförmi Kanale blieben schadenfrei jedoch einiger Schaden Sacculae wurde beobachtet Die Veränderungen wur in vier Kontrolltieren nicht wahrgenommen Sond stabstellung hatte Schalleitungsstorungen in einigen I len zur Folge Hochfrequenzverlust ist zu erwarten w Bestrahlung von 52 mW für 20 Minuten oder langer du das runde Fenster vorgenommen wird

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OPTOKINETIC AFTERNYSTAGMUS AND POSTROTATORY NYSTAGMUS IN SQUIRREL MONKEYS

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(Received February 2? 1977)

Abstract Regardless of whether the direction of optokinetic stimulus matches or conflicts with that of vestibular stimulus if the squirrel monkey subject has a good target pursuing ability the enhancement or inhibition of post stimulatory nystagmus occurs The nature of the stimuli to provoke optokinetic afternystagmus and postrotatory nystagmus are not the same but the similarity of response characteristics and cross interaction strongly suggest the existence of a common neural linkage between the stimulus receptor and reactor

The prime function of the oculomotor system is to precisely pursue the moving target in order to obtain proper visual information In this regard the test for eye tracking activity or optokinetic nystagmus (OKN) test is considered to be natural and appropriate manner to evaluate the oculomotor function

When the optokinetic stimulus ceases and the subject is kept in the dark the eyeball continuously beats to the same direction of that of OKN This is the optokinetic afternystagmus (OKAN) Even though some characteristics are similar for OKAN and postrotatory nystagmus (PRN) (Kornhuber 1962 Takemori 1974 Robinson 1975) many things should be studied regarding the nature and characteristics of this OKAN

Both the PRN and the OKAN may be considered undesirable phenomena to the body because of the fact that those represent prolonged post stimulatory reactions Thus it is suspected that the equilibrium system intends to inhibit those

It has been well known that visual and vestibular systems coordinate each other under natural circumstances and for the purpose of smooth operation of the oculomotor (and body equilibrium) system From this viewpoint this present study was initiated in order to compare the physiological meanings of these two nystagmus and interaction between the PRN and the OKAN

METHODS

The experimental subjects were six randomly selected adult male or female squirrel monkeys with body weight range 500–700 g The monkey was secured in the laboratory built restrainer Undue pressure to the posterior neck region was avoided Subdermal platinum needle electrodes were implanted at the outer canthi and the horizontal eye movement was recorded on the Beckman Dynograph through a DC amplifier All tests were begun about 15 minutes after the administration of amphetamine 0.5 mg/kg

The squirrel monkey was placed at the center of a 60 cm diameter illuminated white cylinder which had 16 vertical black stripes (1 7 cm wide each and equally separated) This cylinder could be rotated 0–200°/sec speed with

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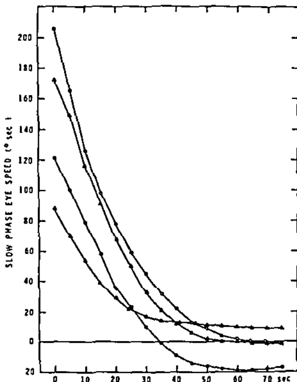


Fig 1 This figure exhibits the average nystagmic slow phase eye speed after 200°/sec st mulus in vertical axis (N 6) along the poststimulus t me course (in horizontal axis) by comparing four experimental situations open circle PRN+OKAN reverse direction open triangle PRN closed circle OKAN+PRN and closed triangle OKAN

0°/sec² constant angular acceleration This tokinetic stimulator provided a whole visual field rotation around the subject When the stimulus speed reached 200°/sec, the illumina tion was instantaneously eliminated

The rotation table at the coaxial center of the optokinetic cylinder could also be rotated at the identical speed with the same angular acceleration When the rotatory stimulus reached 200°/sec the table was stopped (the experimental situation 2) and the illumination was abolished simultaneously (the experimen tal situation 3) However this impulsive cessa tion of rotatory stimulus required about 1 sec to reach 0°/sec, the complete stop The mech anical reason for this was basically due to the mass which had to be stopped

The experimental situation 1 was the illumi nated optokinetic cylinder rotation optokine tic stimulus application and the recording OKN and OKAN

The experimental situation 2 was the subje rotation in darkness rotatory stimulus a plication and the recording of PRN

The experimental situation 3 was the subje rotation in light In other words rotatory sti ulus and optokinetic stimulus were given co comitantly in order to evaluate the coexisti condition of OKAN+PRN

The experimental situation 4 was also subject rotation in light however in this i stance a mirror was installed in a 45° plane front of the squirrel monkey s eyes in order reverse the direction of optokinetic stimulu Therefore, in this experimental situation t direction of rotatory stimulus and that of opt kinetic stimulus conflicted

In this experimental series two steps maximal speeds were studied 100°/sec 200°/sec

Test trials were spaced more than thr days therefore the identical test situation a peared once every two weeks We repeate this test sequence three times in order t evaluate the repeatability of the results

RESULTS

A *Results obtained after 200°/sec stimulus*

Fig 1 exhibits the average nystagmic slo phase eye speed (°/sec vertical axis) declin as a function of time after 200°/sec stimu along the poststimulus time course (sec hor zontal axis) by comparing experimental situ tion 1 OKAN (closed triangle) 2 PRN (ope triangle) 3 OKAN+PRN (closed circle) e perimental situation 4 PRN+OKAN revers direction (open circle)

Experimental situation 1 (OKAN) Th average slow phase eye velocity immediatel after the stimulus cessation was 93°/sec Th inter individual variance was relatively sma initially but this became larger as the nystag mus decayed The 50% response reductio

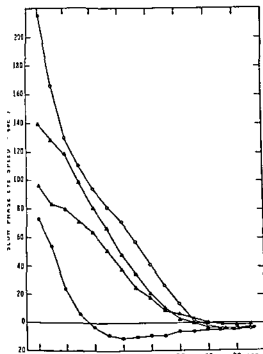


Fig 2 This figure exhibits a representation of strong slow phase eye speed reduction in OKAN+PRN situation, by comparing open circle PRN+OKAN reverse direction and closed circle OKAN+PRN Open triangle PRN, closed triangle OKAN

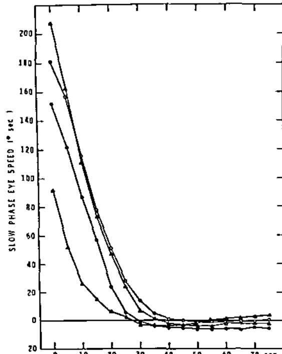


Fig 3 This figure displays a representative case which did not exhibit strong inhibition in OKAN+PRN situation The difference between open circle (PRN+OKAN *reverse direction) and closed circle (OKAN+PRN) is far* less when compared to the situation displayed in Fig 2

occurred about 13 sec after the stimulus cessation

Experimental situation 2 (PRN) The maximum slow phase eye velocity of this PRN immediately after the stimulus cessation was 172°/sec, however it declined rapidly Compared to the OKAN, even though the slow phase eye velocity was high, the inter-individual variance was mimimal

Experimental situation 3 (OKAN+PRN) The initial nystagmic slow phase eye velocity in this condition (PRN+OKAN) averaged 120°/sec The inter individual variance was quite large The response declined to 50% about 15 sec after the stimulus cessation, and the curve asymptoted to a horizontal line after about 50 sec

Experimental situation 4 (PRN+OKAN, reverse direction) The average slow phase eye velocity of this nystagmus response immediately after the stimulus cessation was 206°/sec The 50% response decline occurred around 15 sec after stimulus cessation

Two representative samples of OKAN+PRN cross inhibition (experimental situation 3) are displayed (as closed circles) in Fig 2 (strong inhibition) and in Fig 3 (weak inhibition)

Under the present series of stimulus conditions, the inter individual variance was minimal in the PRN In the OKAN, the inter individual variance was also minimal immediately after the stimulus cessation but it became large along the post-stimulus time course The inter individual variance was large in PRN+OKAN situation, but the PRN was partly inhibited by OKAN The difference in slow phase eye speeds between PRN alone PRN+OKAN was less than the slow eye velocity of OKAN itself (172

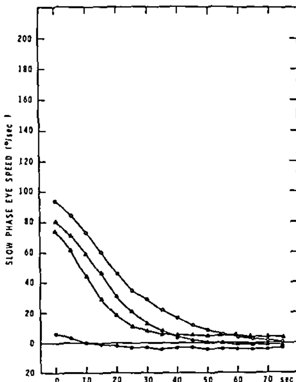


Fig 4 This figure displays the average slow phase eye speed (N=6) after 100°/sec stimuli in vertical axis along the post stimulatory time span in horizontal axis under four different experimental conditions Open circle PRN+OKAN reverse direction open triangle PRN closed triangle OKAN and closed circle OKAN+PRN

sec) The average value of slow phase eye ocity of OKAN immediately after the stim-s was 54% (93/172°/sec) of that of the PRN he average value of slow phase eye velocity in experimental situation 4 (PRN+reverse direction OKAN) was slightly enhanced over that of PRN itself The difference between these two situations was smaller than the difference between the PRN and PRN+OKAN (206–172<172–120°/sec) Nonetheless, the result indicated that the PRN was reinforced by the existence of OKAN even when the direction was reversed

B *Results obtained after 100°/sec stimulus*

Fig 4 demonstrates the average slow phase eye speed (°/sec, vertical axis) plotted as a function of time after cessations of 100°/sec stimuli in four experimental situations

Experimental situation 1 (OKAN) The average slow phase eye velocity of the OKA immediately after the stimulus cessation wa 75°/sec The inter individual variance wa minimal, but subsequently became large alon the post stimulus time course The maximu slow phase eye speed declined 50% about 1 seconds after the stimulus cessation

Experimental situation 2 (PRN) The ave age slow phase eye velocity of this PRN im mediately after the stimulus cessation wa about 80°/sec and the inter individual varianc was minimal The slow phase eye velocit declined 50% about 17 sec, and the curve came parallel to the zero horizontal line abo 50 sec after stimulus cessation

Experimental situation 3 (OKAN+PRN) The average initial value of nystagmic slo phase eye velocity resulting from this PRN OKAN was very low, about 6°/sec The inte individual variance was minimal

Experimental situation 4 (PRN+OKAN reverse direction) The average nystagmi slow phase eye velocity in this reverse direc tion OKAN combined with PRN was 94°/sec Along the post stimulus time course inter in dividual variance became large The slo phase eye velocity declined 50% around 2(sec after the stimulus termination

Results of the OKAN (experimental situa tion 1) and the PRN (experimental situation 2 showed some resemblance, however, th value of slow phase eye velocity of the forme was smaller and the inter individual varianc was larger In experimental situation 3 (OKA +PRN), the post stimulatory nystagmic re sponse was almost totally inhibited When th reverse direction OKAN was combined wi the PRN (experimental situation 4), the aver age value of slow phase eye velocity wa slightly elevated compared to that of the PRN The PRN and the reverse direction OKAN reinforced each other, however, the case t case variance was large as is well displayed i Fig 5 and 6

Fig 7 shows the nystagmic recordings after 100°/sec stimulus under four different stimulu situations from a representative subject

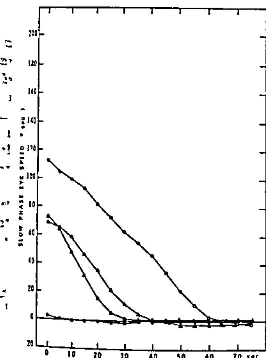


Fig 5 Similar to the situation in Fig 2 this particular subject showed strong nystagmus inhibition in the stimulus condition of OKAN+PRN (closed circle) Open circle PRN+OKAN reverse direction open triangle PRN and closed triangle OKAN

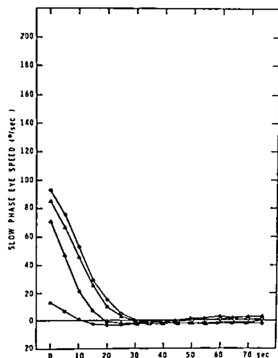


Fig 6 This figure displays less difference between the condition of PRN+OKAN reverse direction (open circle) and that of OKAN+PRN (closed circle) comparing to the results seen in Fig 5

Comparison of slow phase eye velocity of per stimulatory nystagmus elicited by subject rotation in light (experimental situation 3) and by subject rotation in light with a mirror (experimental situation 4) is exhibited in Fig 8 The figure shows clear suppression of slow phase eye velocity under the stressful situation of vestibular visual directional conflict in the latter stimulus situation However, when a mirror was installed in a 45° angular plane in front of the subject s eyes the distance between the eyes and black stripes on white cylinder was elongated and also the visual axis and cylinder surface formed an obtuse angle causing distorted image Even though we have used an identical stimulus speed, the true stimulus speed might have been reduced to about 60% of the original speed The squirrel monkey subject which had the best ability to pursue reverse-direction visual target showed 54% increase to the value obtained in the PRN alone

The above described direction conflicting stimulus situation should cause very unpleasant sensation, and thus also for this reason many of the subjects might not be able to pursue visual target properly Clearly this is the situation of vestibulo-visual conflict An important finding in this part of the experiment was that if the squirrel monkey subject showed good reverse direction OKN even though the two stimuli are direction conflicting and unnatural, the reverse direction OKAN was positively summated with the PRN, and as a result the PRN was enhanced This was the opposite of the results that OKAN and the PRN inhibited each other after the subject rotation in light

The visual tracking ability was v among the different individuals,

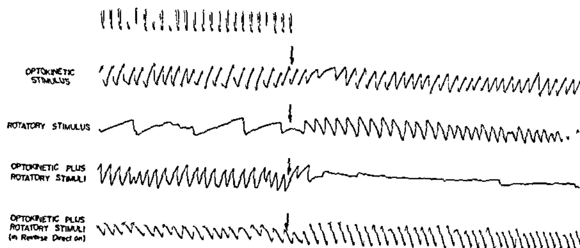


Fig 7 Nystagmus recordings from one representative subject under four different stimulus conditions Arrows indicate the stimulus cessation The maximum 100°/sec

when the system evoked the OKN (regardless of the stimulus direction) the OKAN and PRN showed similar characteristics

C Comparison of two stimulus conditions maximum speed—200°/sec and 100°/sec

The maximum slow phase eye velocity of the PRN immediately after the 100°/sec stimulus, was 46 5% of that which occurred after 200°/ stimulus Therefore, the PRN response dependent on the maximum stimulus ven to the peripheral cupula crista system In contrast, the maximum slow phase eye velocity of OKAN after 100°/sec stimulus was not about 1/2 of that which occurred after the 200°/sec stimulus The reason for this difference was that, when the maximum optokinetic stimulus speed was 100°/sec, all squirrel monkeys could pursue visual stimulus without any difficulty However when the maximum stimulus speed was continuously increased to 200°/sec their maximum ability of persuit was less than 200°/sec speed Furthermore, large inter-individual variance was observed, there were subjects with good pursuing ability and subjects whith poor pursuing ability regarding the maximal stimulus speed they could pursue

The slow phase eye velocity of the OKN immediately before the termination of 200°/sec stimulus was about 70% of 200°/sec in monkey with the best pursuing ability maximum slow phase eye velocity of OKAN immediately after the stimulus mination in this particular monkey was abo 69% of the maximum slow phase eye veloci of PRN Those values obtained from the rel monkey with the poorest pur ity in this group were about 41%, and 45%, respectively The maximum slow eye velocity of OKAN was dependent on eye speed of OKN before the stimulus ces tion, and also was comparable to the eye of PRN

The overall average values indicated th the slow phase eye velocity of OKN ately before the 200°/sec stimulus was about 53% of the stimulus speed slow phase eye velocity of OKAN ly after termination of the 200°/sec st was about 52% of that of PRN If the s phase eye velocity of OKN immediately fore the stimulus termination and stimul speed matched, the maximum slow phase velocity of OKAN immediately after the st ulus termination was almost equivalent to maximum slow phase eye velocity of the

The squirrel monkey subjects could a visual stimulus 100°/sec without

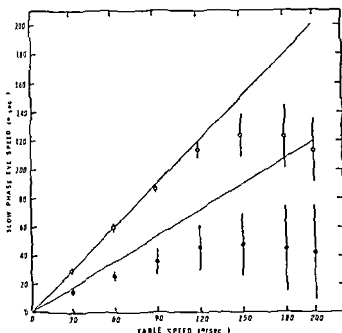


Fig 8 This figure displays the comparison of per stimulatory nystagmic slow phase eye speed elicited by rotatory plus optokinetic (open circle) and by rotatory plus optokinetic reverse direction (closed circle) stimuli *The result shows clear suppression* of the slow phase eye speed under the stressful situation of vestibular visual directional conflict The upper straight line indicates 45° line and the lower straight line is a theoretical representation of 60% of the 45° line Vertical bars indicate standard deviations

trouble The average maximum slow phase eye velocity of the OKAN after 100°/sec stimulus was 75°/sec and the average maximum slow phase eye speed of the PRN after an identical stimulus speed was 80°/sec

The stimulus condition to provoke OKAN *together with* PRN *(the subject rotation in* light) is probably the most natural stimulation *condition to the body The inter individual* variance of the nystagmic response after 200° /sec stimulus in this condition was relatively large, *however, it was not so after the stim* ulus speed of 100°/sec After 100°/sec stimulus, *these two types of nystagmus strongly inhibit* each other and *in most instances the* slow phase eye velocity after the stimulus was less than 10°/sec

When the stimulus is given at the maximum speed of 200°/sec OKAN was much smaller compared to the PRN Therefore the OKAN might not influence the *PRN* and as a result, the difference between PRN vs OKAN+PRN was minimal In this particular condition, the direction of OKAN and PRN were opposite The fact that *OKAN* and *PRN* inhibited each other indi natures of these two types of nystagmus were similar but that these had to have some com *mon neural linkage where the cross inhibition* could take place

Under the most natural conditions, when *the body moves* either actively or passively, *the surrounding whole visual field moves* sim ultaneously In the past it has been empha *sized that the inhibition of* PRN is very much influenced by the visual fixation This present experimental result (particularly after 100°/sec *stimulus) indicated that* if the subject pursued visual target properly, the OKAN inhibited the PRN *in order to assist the nystagmic inhibition* resulting from the visual fixation

The other experimental situation the combination of rotatory stimulus plus reverse direction optokinetic stimulus, was an unnatural situation Comparing this PRN+OKAN (reverse direction) situation with the PRN, the difference in the *slow phase eye velocity* was small after 200°/sec stimulus, however, this difference was a little larger after the stimulus Yet, these differences smaller compared to those f

In this experimental series, the table was rotated with an angular acceleration speed of 1°/sec [2] Under the administration of amphetamine, the majority of squirrel monkey subjects showed slight per-rotatory nystagmus in dark

DISCUSSION

The interaction (coordination or cross-inhibition) between the visual and vestibular systems has been known for a long time Dodge (1923) observed that the visual fixation (or visual input) reduced the nystagmic amplitude during the body rotation with angular acceleration (per-rotatory nystagmus) By utilizing the electronystagmogram (ENG), Ohm (1926) recorded the PRN inhibition by visual fixation These two classic observations indicated that the visual input could control the vestibular evoked oculomotor response, the former had more dominance than the latter did within the body equilibrium system

Mowrer (1935), utilizing the pigeon, found the difference in postrotatory head nystagmus between two conditions, rotation in light and ...n in dark He reported that the OKAN ...ited the post-rotational vestibular nystag- ... In 1937, Mowrer further performed sim- ... experiments in human subjects and reported that the PRN duration after the rotation in light was about 1/2 of that after the rotation in dark (His PRN observation was made in light) This observation was important because of the fact that the visual input affected the vestibular input even after the stimulus termination Guedry et al (1961), and Collins (1968) evaluated the influence of short-term illumination on the vestibular evoked nystagmus or subjective sensation during the angular deceleration, or after the cessation of rotatory stimulus Their observations indicated that the visual input acted in a inhibitory fashion on the vestibular input

The functional coordination between visual and vestibular systems was reported by Fukuda et al (1975) In the rabbit, the vestibular nystagmus was not evoked in dark with ... sec² angular acceleration However, this s... liminal vestibular stimulus enhanced the ... provocation in this animal species

The circular vection (the rotatory sensat... provoked by rotatory optokinetic ... alone) was previously reported by Fis... Kornmüller (1930) and Gurnee (1931) ...ly Dichgans & Brandt (1972, 1973) uti... this phenomenon, successfully ... pseudo-coriolis effect Young et al (1... studied the coordination and discoordinat... between circular vection and vestibular ... lation, and observed the nonlinear rel... of visual and vestibular interations The ci... lar vection observed by Dichgans & B... (1972, 1973) and Brandt et al (1973) co... uted to the understanding of the ... between OKAN and the PRN

The post rotatory subjective sensation ... caused by cupular deviation The degree ... nystagmus and the degree of rotatory ... tion are dependent on the magnitude of t... vestibular stimulus, but are not neces... equivalent to each other When rotated ... light, if the subject can pursue the target ...erly, the provoked OKN should have a ... identical to the table rotation This opt... speed information interacts with the speed ... formation delivered from cupula crista ... tem It is generally accepted that the ... course of the PRN represents the degree ... cupular deviation Because of the fact that ... time course of OKAN and PRN are simil... there is a possibility that the time course ... PRN is also influenced by the central ... discharge mechanism, as Robinson (... pointed out

The vestibular-visual interaction, which ... been investigated through the nystagmic ... sponse or subjective sensation, became a ... finite phenomenon after the establishment ... the direct neuronal recording at the le... vestibular nucleus when only the visual st... lus is given (Dichgans et al , 1973, Leopo... al , 1973, Henn et al , 1974, Azzena et ... 1974) Moreover, after observing the influ...

optic stimulus at the level of the periph- vestibular nerve in an immobilized fish. ke & Schmidt (1970) indicated the exist of an efferent effect at the peripheral level Those neurophysiological ob- confirmed the existence of an ex- y intimate visual vestibular relation-

effects from the visual side can be de- either by visual fixation or OKN Fu- Tokita (1964) reported that, when the direction matches the optokinetical calorically induced nystagmus enhance other whereas when one direction op- the other, it inhibits the other This fact confirmed in subhuman primates in this study Because of the fact that those bulo-oculomotor response and oculo- lomotor response were added or sub- these two functions should possess tain common neural linkage

lowrer (1935 1937) previously suggested direction-dependent relationship between AN and PRN Utilizing a prism he re the direction of optokinetic stimulus the rotation, but failed to observe the ancement even though the *directions of* AN and PRN were the same In our pres t experiment in the squirrel monkeys an was *observed Even though* the ed angular acceleration was minimal (1°) when the visual stimulus and vestibular lus were given in the conflicting direc the vestibular visual conflict occurred d animals failed to pursue *the visual target* However the interindividual dif was large and when the *monkeys* netic target pursuing ability was good *PRN* was enhanced by the OKAN

In the present study we expected that the of PRN inhibition had to be *dependent* the degree of OKN Thus we have used steps of maximum speeds 100°/sec and 00°/sec to compare the effects The inter in ividual variance of optokinetic target pursu ity (maximum) in humans was reported be large (Blomberg 1960) Similarly a large inter individual variance was found in squirrel monkeys

Krieger & Bender (1956) using rhesus mon- keys, reported that the OKAN was inhibited when illumination was given However, when the light was eliminated the nystagmus re- appeared therefore, this behavior of the OKAN was similar to that of the PRN The reduction or abolition of the OKAN after brain stem lesion placement was reported by Shanzer et al (1958) Cohen et al (1973) ob- served the disappearance of the OKAN after bilateral labyrinthectomy All of these ob- servations were done with optokinetic stim- uli alone, and the interaction between the op- tokinetic stimuli and the vestibular stimuli was not studied

From our present observation, the inter in dividual variance of the OKAN, especially re- garding its duration, was relatively large On the other hand, the intra individual repeatabil- ity of OKAN was good The time course of OKAN and that of PRN showed a similar trend This result suggested that the optic and vestibular after-discharge mechanisms were similar Not only their time course but also the slow phase eye velocity of the PRN and OKAN *were similar* immediately after the stimulus cessation when the maximum speed was *equivalent* It was thus reasonable to sus pect that cross inhibition between those two stimuli could exist, specifically when the table was rotated in light and the light was eliminated when the rotation stops This cross inhibition was clearly observed at the maximum speed of 100°/sec When the maxi mum speed of 200°/sec was used however the subjects target pursuing ability could not reach 200°/sec speed and the OKAN was not strong enough to compete against the PRN which was *provoked by the termination* of 200°/sec rotatory stimulus As a result, com- pared to the pure PRN the change *in PRN* in *this situation* was found to be minimal There- fore a correlation was found between the gree of optokinetic

the degree of PRN inhibition

ACKNOWLEDGEMENT

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ZUSAMMENFASSUNG

Wenn der Totenkopfaffe eine gute Fahigkeit erweist ein *Ziel zu verfolgen* dann tritt eine Erhohung oder eine *Hemmung des nachreizenden Ny*
die
kine
Die
nystag_mus und Nachdrehnystagmus hervorrufen sind *nicht gleich* Es ist jedoch eine Ahnlichkeit vorhanden in der Reaktion der Kennzeichen und der kreuzweisen ge genseitigen Beeinflussung die sehr stark auf das Vorhan *densein* von gemeinsamen Nervenverbindungen zwi schen dem Reizempfanger und dem Reizgeber verweisen

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VISUAL-VESTIBULAR INTERACTION UPON NYSTAGMUS SLOW PHASE VELOCITY IN MAN

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act The influence of vestibular and visual (opto-ic) stimuli on the nystagmus slow phase velocity) in man was studied using different combinations sual horizon and/or passive body rotations (velocity zoids) The interactions of combined stimulation evaluated in comparison to pure optokinetic and vestibular reactions The results indicate that retinal e stabilization and vestibular systems simultaneously ate ocular reflexes during passive body accelerations · light

ilts

Exclusive vestibular stimulation by passive body ion in the dark yields a rather low gain of the ves o-ocular reflex (VOR) with respect to acceleration

With optokinetic stimulation the gain depends on rn velocity It is close to unity at velocities below ec and progressively decreases with increasing pat velocity

During passive body rotation in the light (a conco-nt visual and vestibular stimulation occurs) the form he response profile suggests that both visual and ibular inputs contribute to the response Compared pure optokinetic stimulation this results in a better espondence of the SPV and stimulus velocity at er accelerations and velocities

When subjects viewed a surround rotating with con it relative velocity the amount of enhancement or ression of SPV by the additional application of pas body rotation increased with the body acceleration l with surround velocity With small surround velocities l high enhancing body accelerations the SPV may be ter than the relative surround velocity Depressing ves ılar stimuli cause a greater SPV modulation than en ıcing of the stimuli sometimes even reversing the direc n of nystagmus The results indicate an interaction be ·en optokinetic (visual) and vestibular inputs

isual (retinal image stabilization) and ves bular reflexes may initiate eye movements as ıe head is moved freely within a normally lit nvironment Since it is known that both ops, if separately stimulated under optimal onditions, can work at a gain close to unity (Barr et al, 1976, Morasso et al, in prep, Dodge et al, 1930, Mackensen, 1954) the question arises as to how the individual gains are adjusted in cases where both channels could be effective at the same time Interaction between the two reflexes has earlier been demonstrated by a number of authors (Ohm, 1932, Ter Braak, 1936, Jung & Mittermaier, 1939, Jung, 1948, Guttich & Okamoto, 1964) but has never been thoroughly quantified

In this paper we have attempted to quantify the interaction between vestibular and visual (optokinetic) stimuli by examining nystagmus slow phase velocity (SPV) under quasi natural, and artificial conditions, with combinations of mutually enhancing and depressing stimuli, consisting of passive body rotations and visual surround motions The results indicate that the signals created by the retinal image stabilization and vestibular reflex systems may be combined in a weighted sum by the central nervous system to produce a single SPV output This interaction may serve as a basis for the interpretation of clinical data on optokinetic nystagmus SPV in patients with spontaneous nystagmus due to peripheral ves tibular lesions The following paper (Brandt et al, 1978) presents the clinical data

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[2] Presently at Institut für Hirnforschung Zürich

METHODS

Subjects

Four subjects (three male, one female) were selected from a group of 40 students on the basis of their stable slow phase velocity and regular nystagmus frequency in response to constant optokinetic stimulation They had normal vestibular function and were familiar with our apparatus from previous experiments

Apparatus

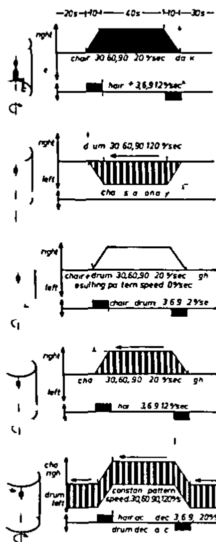
For the experiments, subjects were seated in a chair which rotated about the axis of a surrounding cylindrical drum, 1 5 m in diameter, the inner walls of which were covered with 48 alternating black and white vertical stripes each subtending 7 5 degrees of visual angle (Tonnies, Freiburg) The moving pattern filled the entire visual field Both the chair and the drum could be rotated (independently or coupled) at servo-controlled velocities Constant accelerations which ranged between 3 and 12°/sec² were applied to either chair or drum, or both The commanded relative displacement between chair and drum was verified by means of a photocell attached to the chair and placed facing the stripe pattern Horizontal eye movements were recorded (Jung, 1953) using Beckman silver–silver chloride electrodes After d c amplification, the eye movements and velocity signals from drum and chair were stored on FM magnetic tape for later analysis Calibrations of eye movements were taken from voluntary saccades (four targets, at 15° and 30° left and right) These were repeated several times during each experiment

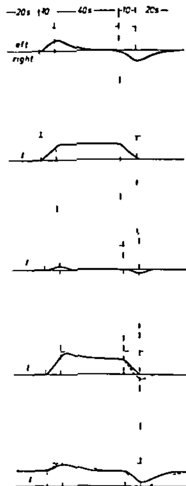
Experimental procedure

Each subject was seated with his head restrained by a head holder and was asked to attentively pursue the stripe pattern With pure vestibular stimulation in the dark the eyes were open The stimuli used are schematically depicted in Fig 1 They were velocity trapezoids each consisting of an initia stationary period, a constant acceleration pe riod (3, 6, 9, 12°/sec² for 10 sec), a constar velocity period (30, 60, 90, 120°/sec for 40 sec) a constant deceleration period (3, 6, 9, 12 sec² for 10 sec) and a final stationary period Stimuli consisted either of (*a*) exclusive cha (body) rotation in the dark (vestibular stimu lus), or (*b*) exclusive drum rotation (opto kinetic stimulus) Suppression of vestibula nystagmus by the presence of a stationary pa tern (*c*) was tested by moving the drum an the chair while they were mechanicall coupled together ("fixation suppression Vestibular and optokinetic stimulation we combined in a natural way (*d*) by rotating th chair (body) in the light ("natural combina tion") The vestibular modulation (*e*) of opto kinetic nystagmus (OKN) in response to constant velocity pattern motion (30 60, 9 120°/sec) was studied by the intermittent ap plication of body accelerations and decelera tions (3, 6, 9, 12°/sec²), which enhanced or de pressed the SPV ("interaction trials) Th combination of four accelerations and f pattern velocities resulted in 16 trials for th experiment After an initial period of pu optokinetic stimulation (drum rotation) las ing 20 sec, subjects were submitted to a 10 se period of chair acceleration from rest in th opposite direction while the drum was d celerated at the same rate, thus keeping th optokinetic pattern speed—relative to the su ject—constant Thereafter for 40 sec the cha was rotated at constant speed while the rel tive velocity between drum and chair remain constant Then a reverse acceleration of cha and drum was applied to reach the origin velocity levels The sequence of the trials w reversed for 2 of the 4 subjects

Data analysis

The eye movements, recorded on tape, we analog to digital converted every 10 msе stored on digital computer disks and analys with a modified Fortran version of the puter program MITNYS II (Allum et a





1 Stimulus combinations of experiments *a–e* and the typical SPV responses (schema)

) The program automatically computed every 10 msec sample the slow phase ve ty of nystagmus and the consecutive 2 sec rage of SPV The program used a linear bration rather than a cubic or sinusoidal bration based on the voluntary saccades ited between targets at 0° 15° and 30° and left Because of the limited storage of the computer (8K IBM 1130) only sec could be analysed at one time Thus ac-

celeration and deceleration periods had to be processed separately Digital plots presented the original nystagmus together with the slow phase velocity time history The values of 2 sec SPV averages employed for this paper were taken from the computer printouts. These values were used for the comp of the average maximum and minimum of our 4 subjects For computations the average time history of SPV the

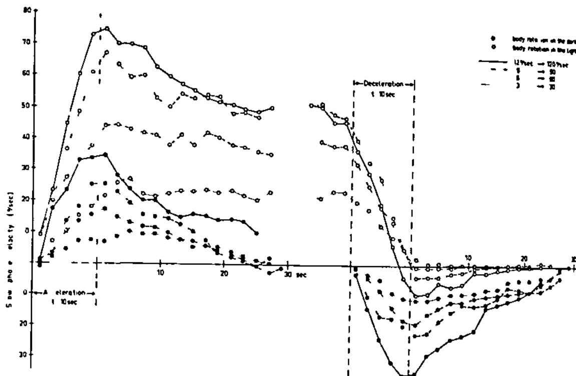


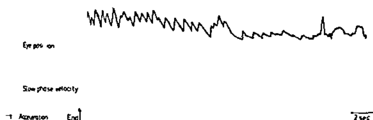
Fig 2 Time course of SPV during pure vestibular stimulation (●) and during natural combination of vestibular and optokinetic stimulation (O) at d fferent accelerat o and corresponding plateau velocities

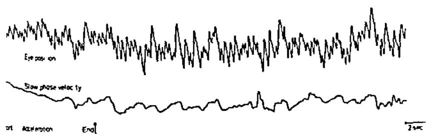
2 sec average values were linearly interpolated with respect to acceleration or deceleration onset

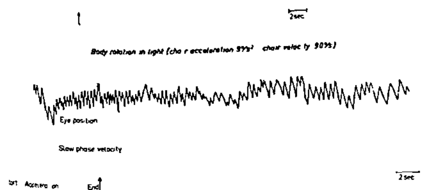
RESULTS

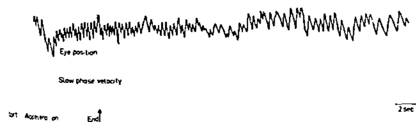
Pure vestibular stimulation

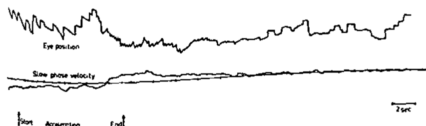
Velocity trapezoids of body rotation in the dark (Fig 1*a*) caused per and post rotatory vestibular nystagmus, the average time course of which is depicted in Fig 2 Fig 3*a* shows a representative original recording with the corresponding computer plot The increase of SPV during the 10 sec acceleration period is well fitted by a straight line (average coefficient of determination (r^2) is 0 92, standard deviation (S D)=0 15) With pure vestibular stimulation, the SPV always progressively lagged behind the increasing chair velocity as the vestibulo-ocular reflex (VOR) long time constant became more effective The vestibular response latency was included in the regression calculations The accelerations of the chair (3, 6 9, 12°/sec²) caused an increase i SPV of 1 2, 2 2, 2 9 and 4 0 degrees/sec² re spectively The ratios between the corre sponding values were employed as a measur of vestibulo ocular reflex gain (0 41 0 37 0 32, 0 34) When the decay effect of the ves tibulo ocular reflex long time constant is ac counted for, the true acceleration gain is 35 greater than the value obtained by linear re gression techniques The decay can be ob served after the end of acceleration but o course it is effective during the acceleration period too Maximum SPV (Fig 4) is reached at an average of 1 1 sec after the end of ac celeration This latency of the maximum SPV tended to decrease with increasing accelera tions The decline in SPV during steady chair rotation was fitted by a single exponential function which has an associated average time constant of 15 2 sec (S D =7 2) Deceleration of the chair elicited a nystagmic response in the opposite direction which was symmetrical to the response during the acceleration period











3 Original recordings of nystagmus with the cor onding computer plots from one subject (*a*) pure bular stimulation (*b*) pure optokinetic stimulation ronounced vestibular nystagmus despite the presence of a stationary surrounding pattern (*d*) acceleration (*e*) deceleration phase during body rotation in th (natural combination note reversal of nystagmus tibular stimulus)

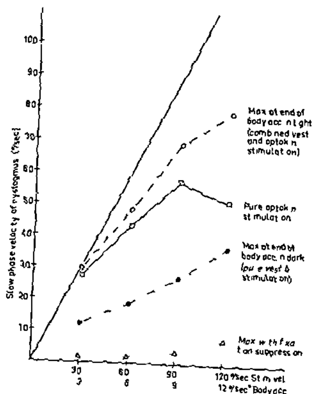


Fig 4 Averages of the four subjects for maximum SPV

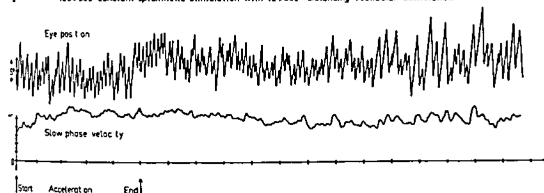
... simulation by body rotation *in the dark* (●) and averages of maximum SPV *during the fixation* suppression experiment (Δ)

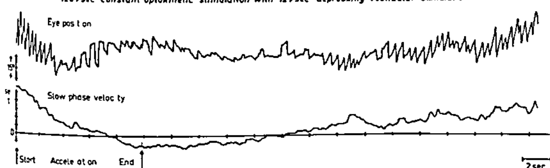
Pure optokinetic stimulation

With full field optokinetic stimulation elicited by rotating the striped drum around the subject (Fig 1*b*), SPV closely matched stimulus speed up to 30°/sec, for all accelerations tested except 12°/sec² The gains for the SPV during the acceleration period were 0 78, 0 63, 0 68, 0 67 Periods at the end of the acceleration, where the SPV had saturated for higher pattern velocities, were excluded from the regression calculations At higher pattern velocities, the SPV was progressively less than the stimulus speed The maximal steady state SPV was reached at a pattern velocity of approximately 90°/sec and decreased at higher speeds (Fig 4) It showed considerable interindividual variability (range 52–71°/sec for 90°/sec stimulus, among our subjects) The maximum OKN gain (maximum values of SPV divided by stimulus speed) decreased from speeds of 30°/sec to a value of 0 5 at The velocity gain (average values 0 9 0 63, 0 43 for 30, 60, 90 120°/sec respe was maintained throughout the constan ity period Maximum SPV of the inc trials was normally reached after the te tion of the acceleration Some waxi waning of the SPV occurred during th stant velocity period (Fig 3*b*) The st deviation associated with this var amounted to 6 6, 4 4, 5 8, 7 9% of th vidual average SPV, respectively, for th stimulus velocities tested During the leration phase, the SPV remained at the acquired during constant velocity stimu and only decreased when the stimulus was close to the level of the SPV Onc SPV decreased, its gain during the dec tion period was 0 96, 0 82, 0 87, 0 85 (in of increasing deceleration) thus being gr than the acceleration gains

Vestibular stimulation in the presence of a stationary optokinetic pattern (fixation suppression)

When chair and drum mechanically cou together are accelerated at the same rate (1*c*) the use of the retinal stabilization of striped pattern which is stationary with spect to the chair dramatically decreased nystagmus response caused by the vestibu stimulus The effects of suppression lead t highly variable maximum SPV which amou on average, to 13% of the maximum SPV d ing pure vestibular stimulation The variabil of the SPV hampered any assessment of SI decay time constants For the accelera we used, stable fixation in some cases could recovered before or just after the end of th acceleration period but in others nystagmu also persisted up to 20 sec after the end of a celeration An original recording of a pa ticularly pronounced nystagmus elicited by body acceleration of 9°/sec² in the presence c a stationary pattern is shown in Fig 3*c*





5 Original recording of nystagmus and corresponding computer plots during a 120°/sec constant velocity optokinetic stimulation combined with (*a*) 12°/sec² enhancing and (*b*) 12°/sec² depressing vestibular stimulation (note the increase in SPV and frequency of nystagmus in (*a*) and the reversal of nystagmus in (*b*)

Natural combination of vestibular and optokinetic stimulation

When velocity trapezoids are applied to subjects seated in a rotating chair while the illuminated drum is kept stationary (Fig 1*d*) vestibular and optokinetic stimuli are combined in a quasi natural way (Fig 3*d*) The gains of the SPV during the acceleration period (0 72, 0 64, 0 74, and 0 73) did not differ significantly from those obtained by optokinetic stimulation (excluding saturation effects) The average gain factors with respect to stimulus velocity, as calculated from the maximum SPV are 0 99, 0 80, 0 76 and 0 66 for the four velocities tested Thus increasing vestibular stimulation results in a better transient response for all velocities compared with optokinetic stimulation Nonetheless, the maximum SPV gain factors were less than one would have expected from the sum of the corresponding individual gains for optokinetic and pure vestibular stimulation

The maximum SPV (2 sec average value) as for pure vestibular stimulation, was reached after termination of the acceleration period The delay between acceleration and the maximum SPV decreased with increasing acceleration (5 6 sec, S D 1 6, 3 0 sec, S D 1 9, 0 4 sec, S D 0 9, 0 3, S D 1 0 for the four accelerations 3–12°/sec² and the corresponding velocities 30–120°/sec tested) After its peak, the SPV decayed to an average plateau which, 23 sec after the end of acceleration, had a velocity gain of 0 72, 0 68 0 53, and 0 40 The difference with respect to the optokinetic gain measured at the same time is statistically significant The time const the single exponential fit to the de

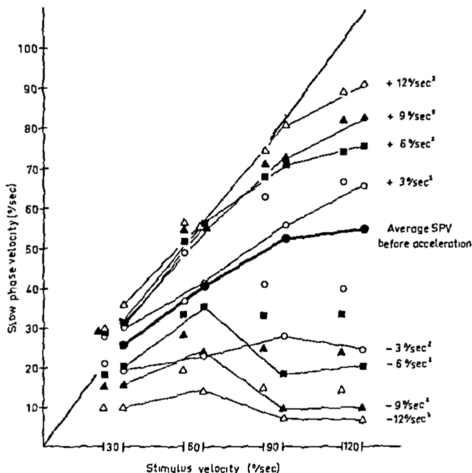


Fig 6 Comparison of obtained and predicted maximum and minimum SPV values elicited by additional vestibular ulation during constant velocity pattern motion The obtained with the combination of each pattern city and each acceleration is represented by 2 values by identical symbols The value further on the right hand side for any given stimulus velocity shows the experimentally obtained SPV The experimentally c tained values are connected by lines The value further the left shows the SPV predicted on the basis of the f mula given in the algebraic summation hypothesis T amount of SPV modulation can be obtained by compar the maximum and minimum SPV values with the SPV l fore acceleration onset

sec, S D 7 7) is not significantly less than the time constant obtained for body rotation in the dark

With the onset of deceleration, the vestibular component of the stimulus immediately depressed the SPV even though the relative motion of the optokinetic pattern, on the basis of the OKN deceleration response, would tend to maintain SPV at the plateau level (Fig 2) The decrease of the SPV (−2 2, −3 6, −4 8, −5 9°/sec²) during deceleration, however, was more shallow than the increase of the SPV during the acceleration phase (2 2, 3 9, 6 7, 8 1°/sec²) A deceleration of 3°/sec², caused nystagmus to cease once the rotating chair w at rest With decelerations equal to or great than 6°/sec², the direction of nystagmus fr quently reversed (Fig 3*e*) This was seen in 4 subjects with a deceleration of 12°/sec² Th reversal may even start before the end of th deceleration period The amount of po rotatory nystagmus differed widely for o subjects Subjects with high SPV during th constant velocity phase showed no or on little nystagmus reversal at the end of th deceleration phase With slow SPV prior deceleration subjects tended to show stro postrotatory nystagmus A persisting inte

ion between the optokinetic and vestibular tem became apparent after the chair had me to rest During this period the reversed stagmus decayed to zero velocity with an erage time constant of 6 7 sec (S D 1 0) ch is shorter than the vestibulo-ocular re- x time constant as determined by body rota- n in the dark

stibular stimulation in the presence a constant velocity pattern

this group of experiments (Fig 1*e*), after citing optokinetic nystagmus by rotating the nped drum, subjects were accelerated in the pposite direction This resulted in an en ancement of the SPV, even though the drum as decelerated at the same rate, thereby eping constant the relative surround motion nd therefore, the optokinetic stimulus speed ee original recordings in Fig 5*a*) On re ersing the procedure by decelerating the sub- cts, SPV was depressed (see Fig 1*e*) Com lete suppression of OKN or even reversal in ystagmus direction was observed in 2 of the ubjects with depressing accelerations of 9 and 2°/sec and pattern velocities of 90 and 120°/ ec (Fig 5*b*) With suitable stimulus condi ions (30°/sec pattern velocity and 12°/sec² estibular acceleration), enhancement may ead to a SPV that over several seconds (aver- age 7 0, S D ±4 7) exceeded pattern speed by a few degrees per second This overshoot re- versed the direction of retinal image motion It s possible for calibration errors in eye move ments to cause such small differences This artifact, however, was excluded as a possible cause when 6 additional subjects observed a retinal after image motion faster than the pat tern velocity Normal optokinetic stimulation yields an after-image motion slower than the stimulus velocity

Both the rate of change in SPV during the acceleration phase and the maximum SPV in creased with vestibular acceleration rate and the optokinetic pattern velocity Fig 6 shows the averages for the maximum and minimum SPV for different accelerations and decelera tions combined with different pattern veloc- ities To evaluate the amount of modulation, the mean SPV before acceleration started is also shown The figure clearly demonstrates that the extent of modulation increases not only with the body acceleration but also with higher pattern velocities, and that, on the average, depressing vestibular stimuli have greater effects than enhancing ones The max- imum SPV was reached at an average of 1 2 sec after the end of acceleration, however, this delay varied considerably (S D 5 1) Occa- sionally SPV peaked several seconds before the end of acceleration The minimum SPV for depressing stimuli occurred, on average, 3 4 sec after acceleration had ended

The single exponential fit of the SPV decay after the SPV maximum during depress and enhance trials has an average time constant of 15 9 sec For the trials using small accelera- tions and small pattern velocities, it was not possible to assess the time constant, because of spontaneous fluctuations in the SPV from which a clear acceleration related SPV modu lation was difficult to discriminate Because of the small difference of this time constant com pared with that for pure vestibular stimulation we concluded that, at least for high accelera tions and high pattern speeds, the time course of the vestibular modulation of OKN is deter- mined by the dynamics of the vestibulo-ocular reflex

DISCUSSION

The aim of this study was to determine the interactions of two reflex systems, the opto- kinetic (retinal image stabilization) system and the vestibulo-ocular reflex (VOR) which con verge to produce nystagmus as a common response The experimental conditions were arranged to activate either reflex alone or both together The data from the pure vestibular and the pure optokinetic trials served as basis for the evaluation of the trials, w both reflexes interact

The results of the vestibular stim

passive body rotation in the dark are in agreement with the findings of Brown & Crampton (1964) The gain obtained with trapezoid velocity profiles does not differ significantly from gain values (0 4–0 6) obtained when subjects are rotated sinusoidally in the dark at a frequency of 0 1 Hz (Meiry, 1971, Sugie & Melvill Jones, 1971, Barr et al , 1976) All these studies, including ours, indicate a low gain for the passive VOR at low frequencies In contrast, the passive VOR gain rises to unity for high frequency rotation of 3 Hz (Benson, 1970) or sharp transients (Barr et al , 1976) Thus the low value of the VOR obtained with passive head movements may not be a functional deficit unless active head rotations have significant components at low frequencies The dependency of the optokinetic nystagmus gain upon pattern velocity found in the pure optokinetic experiments agrees with earlier findings of Gruttner (1939), Mackensen (1954), Honrubia et al (1968) and Dichgans et al (1973) An increasing mismatch between stimulus and eye velocities appears in a similar range for pursuit movements (Dodge et al , 1930) This correspondence between optokinetic and pursuit systems, apparent in the ...ar frequency responses and latencies to changes in stimulus velocity, has been ...ised by Yasui (1974)

VOR-suppression

Our results, for conditions when the visual surround rotates with the subject show that the VOR SPV response is not simply cancelled by the presence of a stationary pattern The irregularity of the response suggests that the degree of suppression is dependent on the attention paid to the stationary contrasts Our experiments with a stereotyped visual surround, with numerous equal contrasts possibly being fixated on, are not identical with experiments using one small fixation mark Our vestibular stimulus is not to be compared with repetitive sinusoidal rotations because of their higher predictability Experiments with sinusoidal stimulation show a much better VOR suppression that is weakest above 1 Hz when the VOR frequency response increases (Barr et al , 1976, Benson 1970) and the optokinetic frequency response decreases (Fender & Nye 1961, Yasui, 1974)

Natural combination and interaction trials

In the natural combination experiments and in the interaction trials the VOR and the retinal image stabilization reflex are both activated Three possibilities of how both reflexes when active at the same time, could contribute to the nystagmus response have to be taken into account a switching mechanism a weighted summation, or an algebraic summation

Switching Hypothesis The switching hypothesis (Waespe & Henn 1977) assumes that either the visual or the vestibular input is causing the SPV, the activity from the other input being suppressed From the SPV response observed in the natural combination experiment we conclude that a switching cannot be the underlying mechanism The higher gains during the acceleration phase compared with pure vestibular stimulation suggest a contribution of the visual input Also SPV at the end of body accelerations in the natural combination experiment is faster than that observed for pure optokinetic stimulation The decline of SPV after the end of acceleration in the natural combination experiments occurs with a time constant similar to the vestibular decay and indicates that the vestibular input contributed to the response A further argument against the switching hypothesis is given by the vestibularly induced modulation of SPV in the ' interaction trials which should be absent if a switching mechanism were active The observed SPV profiles can be explained on the basis of the two other hypotheses which suggest different concepts of how sensory signals are combined at premotor structures to produce a single SPV output

Weighted Summation During body acceleration in the presence of a stationary visual surround retinal image stabilization reflex

VOR are simultaneously active With this ' of combined stimulation the question ses as to the input controlling the retinal age stabilization reflex In contrast with the ebraic summation hypothesis discussed er the weighted summation hypothesis as- that the input controlling the opto- c contribution of the retinal image stabil on reflex is proportional to surround ve , Testing this assumption by adding the 's obtained during separate optokinetic vestibular stimulation shows that the ef- ts of the combined stimulation are not pure- additive, especially during acceleration when the added gains for separate mulation are, on the average, 47% greater an the combined stimulation gains One ex ion for the observed effects could be a gain modification of either one or both lex signals occurs before they are com ined i e a weighted summation, so that a PV exceeding the stimulus speed is pre- ented A similar non additive result for the PV has been observed in a non foveate ani l the rabbit, using sinusoidal stimulation & Collewijn, 1974)

The scaling down of the vestibular input ld be elicited by the presence of retinal ,, whereas the optokinetic input to the could be reduced by the presence of body ation From the natural combination experi nts one may conclude that the reduction of he optokinetic gain induced by the vestibular us is relatively minor In these experi ents the SPV at constant velocity body rota ion progressively decreases with ceasing ves ibular excitation to equal the SPV during pure okinetic stimulation

On examining the results for interaction trials (Fig 6) an idea can be obtained of how the vestibular weighting factors may change with stimulus magnitude As relative stripe velocity is increased the vestibular weighting factor for the same vestibular stimulation would increase i e the optokinetic input would influence the vestibular gain weighting

The advantage of the increase in the weighting factors with stimulus magnitude is apparent from the body rotation in the light trials With separate stimulation the gain of each reflex tends to decrease with stimulus acceleration If, however, the weighting factor of each reflex in the weighted summation on combined stimulation were to increase with stimulus magnitude, the effect of gain decrease on the separate reflexes would be minimized and a more linear response result In fact, acceleration gains in the natural combination experiments are constant throughout the accelerations tested

The question arises as to whether retinal error velocity alone is the controlling input to the optokinetic component of the SPV during natural stimulation and the interaction trials Two of our results indicate that this is probably not the case Firstly, the enhancement and depression of SPV occurs for periods of several seconds during the interaction trials Secondly, the time constant associated with the decay of SPV during the natural stimulation constant velocity period is close to that corresponding to pure vestibular stimulation A priori, one would have expected the retinal image stabilization reflex, from its known response latency to error velocity (Rashbass, 1961, Robinson, 1965), to have compensated for the retinal error after its latency (100–200 msec) in these two experiments These results, however, can be explained if one assumes that surround velocity is represented in the central nervous system and is controlling the optokinetic contribution to the SPV This representation would consist of a positive feedback of eye velocity added to the retinal error velocity signal In this case one should assume that the steady state gain of the eye velocity signal and the retinal error velocity signal when summed are less than unity, thus explaining the reduced pursuit tracking ability above 25 to 30°/sec (Young, 1971)

With the representation of surround ve' in the central nervous system as an SPV, the mechanism of depression of SPV for the

be explained For the enhancement trials the vestibular signal together with the positive feedback of eye velocity seems to override any correcting influence of the retinal error velocity signal The overshoot of eye velocity continued for as long as the vestibular signal reinforced by positive feedback exists The enhancement is greater with increasing optokinetic stimulation magnitudes because of the increase in the weighting factors of the vestibular reflex contributing to the total SPV response Similarly for the depression of SPV by the vestibular stimulation the effect of positive eye velocity feedback maintains the depression The eye velocity signal and the vestibular signal are greater than the correcting signal proportional to retinal error velocity

Algebraic Summation At the onset of the natural combination experiments the vestibularly induced SPV is thought to decrease retinal slip and thereby the optokinetic input after a short latency of a few milliseconds, before eye movements on the basis of the optokinetic stimulation have been elicited (latency 100–200 msec, Robinson, 1965) This leads to the assumption that the optokinetic input ht be the difference between surround city and a vestibularly induced SPV component The vestibularly induced SPV component is assumed to be identical with the SPV response to pure vestibular stimulation in the dark by the respective chair acceleration On the basis of these assumptions the SPV during the combined vestibular and optokinetic trials was predicted from the SPV observed during separate optokinetic and vestibular stimulation using the following formula

D=drum velocity

C=chair velocity

$D-C$=optokinetic surround velocity i e drum vel −chair vel

g_v=gain during pure vestibular stimulation

g_vC−SPV during pure vestibular stimulation= vestibular component

$(D-C)-g_vC$=effective optokinetic input i stimulus velocity minus VOR induced SP

g_0=velocity dependent gain as determine during pure optokinetic stimulation

$g_0((D-C)-g_vC)\approx$OKN component of SPV expected from the effective optokinetic input on the basis of the velocity dependent gain observed during pure optokinetic stimulation

$g_0((D-C)-g_vC)+g_vC$=predicted SPV i summation of the optokinetic and the vestibular component

For the trials with vestibular decelerations t signs for the vestibular component had to reversed

The predicted maximum SPV (average ! our 4 subjects) in the natural combination experiments are 28 4, 51 8, 71 2 and 89 4°/sec the four accelerations and velocities To compare them with the observed maximum valu (29 5, 48 5, 68 6, 78 7) we used the ratios (observed value/predicted value) which are 1 0 93, 0 96, 0 88

Although the optokinetic stimulation preceded the vestibular stimulation in the "interaction trials" the same method of predict the maximum SPV from separate vestibular and optokinetic stimulation was used beca the retinal image motion was also changed these experiments by the vestibular stimul Fig 6 compares the observed SPV values the expected maxima For enhancing stimu tion the observed values are on the aver 0 6°/sec higher than the expected values

Of particular interest is the finding of a S faster than the stimulus when a 12°/sec² tibular acceleration was combined with a sec pattern motion A priori one would h assumed that the reversed retinal image tion, elicited by the SPV faster than the tern motion, should have decreased the S via the optokinetic input after a latency about 100–200 msec The reason for the served overshoot of SPV over the stim speed for several seconds could be long las effects of the preceding optokinetic stim

t summed with the VOR Such long lasting ·cts have been demonstrated by Ohm 1) Mackensen et al (1961), Brandt et al 74) Cohen et al (1977) as optokinetic mystagmus (OKAN) A tonic modulation the vestibular neurons discharge rate that lasts the visual stimulus may be the neuro-ysiological basis (Dichgans & Brandt, 1972 hgans et al 1973, Henn et al , 1974, Allum al , 1977) for these phenomena

In trials with depressing vestibular stimula n the hypothesis assumes that the vestibular mulus elicits a hypothetical SPV opposite to optokinetic SPV Thereby the difference tween the vestibular SPV and the opto-netic pattern velocity becomes greater than e actual pattern velocity Thus the OKN un is decreased because of its velocity de ndency Nevertheless the observed mini um SPV values are on the average 7 2°/sec ower than the predicted SPV (Fig 6) A pos ble explanation could be that a storage echanism in the brain stem, the activity of hich gives rise to optokinetic afternystagmus nd is necessary for a high SPV of OKN with gh pattern velocities (Cohn et al , 1973), is ischarged by the vestibular stimulus to the pposite side The indirect effect of the dis harge of this OKAN storage mechanism on OKN–SPV, combined with the direct effect of he vestibular stimulus to the opposite side ould account for the strong inhibition we ob-erved

Our experiments may be the basis for the understanding of clinical data on OKN abnor nalities in patients with vestibular lesions These data will be discussed in the subsequent paper by Brandt et al (1977)

ACKNOWLEDGEMENT

We thank Mrs U Rommelt for Assistance with graphics

ZUSAMMENFASSUNG

Der Einfluss vestibularer Beschleunigungsreize auf die Geschwindigkeit der langsamen Phase (SPV) des optokinetischen Nystagmus (OKN) wurde mit versch edenen Kombinationen von Umwelt und passiven Korperrotationen untersucht Zum Vergleich dienten rein vestibularer und rein optokinetischer Nystagmus

Ergebnisse

1) Wahrend rein vestibularer Reizung im Dunkeln ergab sich ein Verstärkungsfaktor 0 3–0 4 für den vestibulo-okularen Reflex im Verhaltnis zur Beschleunigung

2) Während rein optokinetischer Reizung hangt der Verstarkungsfaktor von der Reizmustergeschwindigkeit ab Bis zu Geschwindigkeiten von 30°/sec ist er nahezu 1 und nimmt dann mit zunehmender Reizmustergeschwin d gkeit ab

3) Wurde die Versuchsperson im Hellen bei stationarem Horizont beschleunigt (naturliche Kombination vestibularer und optokinetischer Reize) so führte die zusatzliche vestibulare Reizung bei hohen Reizmuster geschwindigkeiten im Vergleich zu der unter 2 beschrie benen Versuchsanordnung zu einer schnelleren SPV und damit zu einer besseren Ubereinstimmung zwischen SPV und Mustergeschwind gkeit

4) Bei konstanter optokinetischer Reizmustergeschwin digkeit nahm die SPV Modulation durch intermittierende zusatzliche vestibulare Reize mit der Starke der Be schleunigung *und* für zunehmende Reizmustergeschwin digkeiten zu Bei niedrigen Reizmustergeschwindigkeiten führten starke Beschleunigungen dazu dass die SPV fur mehrere Sekunden die Reizmustergeschwindigkeit uberstieg Den OKN hemmende vestibulare Reize verur sachten eine starkere Modulation der SPV als aktivie rende Reize Hemmende Reize konnten den OKN unter drucken und vorubergehend einen vestibularen Nystag mus in Gegenrichtung auslösen Die Ergebnisse lassen auf eine Interaktion von optokinetischen (visuellen) und vestibularen Einflussen schliessen

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QUANTIFICATION OF VESTIBULAR COMPENSATION IN UNILATERAL MENIERE'S DISEASE

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tract The main parameters of the vestibulo-ocular (i e the gain or reflectivity the long time constant semicircular canals the time constant of the neural transduction and that of the central integrator) were computed from the post rotational tagmic responses of unilateral Meniere s patients mparison with normal subjects showed a decrease of first two parameters both in CW and CCW responses significant changes were observed in the remaining ers Probably due to a process of central com sation a high level of symmetry was observed mainly ing patients

recent years, vestibular induced nystagmus been used to identify the parameters of thematical models of the vestibulo ocular (Schmid et al , 1971, Mira et al , 1975*a* Schmid, 1975) In particular the gain or flectivity, the long time constant of the circular canals, the time constant of the chano-neural transduction, and that of the leaky integrator were estimated with fficient accuracy to discriminate between and pathological situations (Mira et al 75*b*)

Nevertheless, this approach has not yet used to examine a specific pathology in systematic way Meniere s disease seems to e a good experimental model for this pur Actually, it is generally accepted that leniere s disease is caused by a distention of he membranous labyrinth produced by an en hydrops (Hallpike & Cairns, 1938, Altmann & Kornfeld 1965, Gussen, 1973) Therefore the parameters of tagmic response related to the dynamics of the cupula endolymph system are likely to vary in Meniere's patients vis a vis normal subjects On the other hand the critical im balance between the labyrinths due to uni lateral Meniere's disease may induce a process of central compensation Thus in longstand ing Meniere s patients parameters other than peripheral may result varied

The objective of this work was to verify these predictions by using a model approach to quantify the changes in the nystagmic re sponse of Meniere's patients vis a vis a normal population

Rotatory tests (post rotational) were ad opted Actually in this case the relationships among stimulus, cupula deflection and ny stagmus can be precisely defined in mathe matical terms (Jones & Milsum, 1965, Sugie & Melvill Jones 1971, Schmid et al 1971, 1975, Barnes & Benson 1973, Schmid & Lardini, 1976) By contrast some uncertainty exists on the relationships between caloric stimulations and cupula deflections The same caloric stim ulus may produce varying effects on the cu pula endolymph system according to the thermal characteristics of the non homogene ous ear structures through which heat flows

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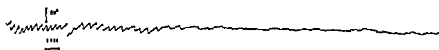




Fig 1 Clockwise (CW) and counterclockwise (CCW) post rotational nystagmus of a patient suffering from a 3-year old Meniere s disease

The mathematical models so far proposed (Steer, 1967, Baertschi et al , 1975, Bock & Bromm, 1977) are the results of heavy simplifications Moreover, in a recent theoretical and experimental determination of vestibular dynamics in caloric stimulations, Baertschi et al (1975) concluded that the long-time constant of the semicircular canals cannot be determined from a caloric test since it is masked by dynamics of heat transmission

Rotatory tests have been considered of limited value in diagnosing Meniere's disease since they give no hint as to which labyrinth is diseased (Stahle, 1968) Caloric tests have been preferred However, it should be noted that the side of the lesion can be equally or even better recognized on the basis of the clinical history of the patient or from audiometric findings (Hedgecock, 1968) From a clinical point of view, it is probably more interesting to test the degree of dynamic balance of the vestibulo ocular reflex response acquired by the patient in the everyday life, following processes of central compensation To this aim, caloric tests are completely inadequate

MATERIAL AND METHODS

Two groups of 25 normal subjects and 15 patients with unilateral Meniere s disease were examined Both groups were submitted on the same day to a clockwise (CW) and a counter clockwise (CCW) post-rotational test

All Meniere's patients had a typical history of disease, with a progressive hearing impairment, tinnitus and attacks of vertigo with nausea and vomiting Audiological examination showed in all cases a unilateral perceptive hearing loss of various degree, with flat or ascending curve and recruitment measured by the method of Fowler and/ or Metz The duration of the symptoms ranged from 1 to 15 years Examination of Meniere's patients was performed in vertiginous free intervals and special care was taken to ensure that no signs of labyrinthine imbalance were present and no pharmacological treatment was undertaken

Horizontal nystagmus was recorded in total darkness by means of two bitemporal silver-silver chloride skin electrodes (Beckman Type 3503) with a ground electrode placed on the forehead The electrodes were connected to a d c amplifier (Beckman Dynograph Type R), and chair velocity and nystagmus were displayed simultaneously on a polygraph The output of the amplifier was also coupled to a four-channel FM tape recorder (Ampex PR 500) After A/D conversion, ENG data were processed by means of an interactive program

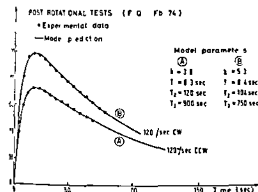


Fig 2 Diagrams of the slow cumulative eye position (SCEP) obtained from the responses in Fig 1 The values of the model parameters computed through a best fitting procedure are given in the upper right corner K gain of the vestibulo-ocular reflex T_1 long time constant of the semicircular canals T_2 time constant of the mechano-neural transduction T_3 time constant of the central neural leaky integrator

(Anzaldi & Mira, 1975, Anzaldi et al , 1977) and the diagrams of the slow phase cumulative eye position (SCEP) were constructed

These diagrams were then fitted by using the following transfer function between the Laplace transform of SCEP and head velocity (Mira et al , 1975)

$$\frac{\text{SCEP}}{\Omega}=\frac{KT_2T_3s^2}{(1+sT_1)(1+sT_2)(1+sT_3)} \qquad (1)$$

where

K = gain of the VOR,
T_1 = long time constant of the lateral semicircular canals,
T_2 = time constant of the mechano neural transduction,
T_3 = time constant of the central neural leaky integrator,
s = Laplace variable

The parameters of the transfer function (1) were estimated by using Marquardt's algorithm (1963) as described by Lardini et al (1975)

Automatic data processing was performed by a general purpose minicomputer (LABEN 70 with 16 k, 16-bit words and 1 35 µsec cycle time) equipped for the graphic presentation of the output (display terminal Tektronix 4010)

In spite of the special care taken in recording and processing nystagmus, the responses of 5 Meniere's cases were so confused by artifacts that they had to be discarded

All the examined patients were also submitted to caloric tests Their nystagmic responses were recorded but not processed by computer

RESULTS

An example of CW and CCW post rotational nystagmus of a patient suffering from a 3 year old Meniere's disease is given in Fig 1 The symmetry of the two responses denotes a high degree of compensation With respect to normal subjects, there is a marked reduction of the response in terms of both beat amplitude and nystagmus duration These observations are quantitatively confirmed by the values of the model parameters computed from the diagram of the SCEP shown in Fig 2 Actually, the gain K computed from the CCW response, corresponding to utriculopetal cupula deflection in the diseased semicircular canal, is about the 70% of the gain computed from the CW response Both the gains are reduced by about 50% with respect to mean values in normal subjects No appreciable difference exists between the value of the time constant T_1 computed from the two responses In this case too a reduction of more than 50% is observed with respect to normal subjects (Schmid et al , 1971, Malcolm, 1973) This finding is in good agreement with the short duration of the primary phase of the response in Fig 1 The remaining parameters T_2 and T_3, differ by about 15% when computed from the two responses Due to the lack of accuracy attending the estimation of these parameters, in particul T_3 (see Appendix), the reported differe cannot be considered as significant. over, the computed values of T_2 a within the range of normality

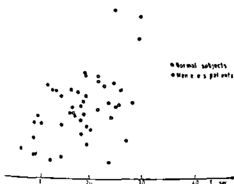
Table I

Patient	Age (years)	Duration of disease (years)	Mean hearing loss in dB[a]	Diseased side	Amplitude (°/sec) and direction of the post rotational test	Parameters of the ENG response: number of beats of the 1st phase	duration of the 1st phase (sec)	peak velocity (°/sec)
B U	42	2	R 10	L	120 CCW	33	15	34
			L 75		120 CW	40	16	18
D V	38	1	R 38	R	120 CCW	45	30	90
			L 28		120 CW	60	35	85
F A	33	3	R 57	R	90 CCW	19	20	47
			L 6		90 CW	58	30	33
F Q	58	3	R 52	R	120 CCW	38	22	47
			L 14		120 CW	35	21	70
M G	67	2	R 70	R	150 CCW	86	33	60
			L 33		150 CW	63	25	100
M A	41	4	R 10	L	120 CCW	60	30	70
			L 42		120 CW	47	28	19
L C	34	2	R 72	R	120 CCW	63	34	20
			L 17		120 CW	53	40	24
P A	56	2	R 27	L	120 CCW	77	45	75
			L 66		120 CW	43	37	37
S C	60	15	R 96	R	120 CCW	44	28	35
			L 57		120 CW	34	20	63
V C	56	4	R 51	R	120 CCW	69	41	28
			L 28		120 CW	42	31	75

[a] Computed at the frequencies of 250 500 1 000 2 000 and 4 000 Hz

Table II

Patient	Diseased side	Amplitude (°/sec) and direction of the post rotational test	SCEP Parameters: K	T_1 (sec)	T_2 (sec)	T_3 (sec)	K/T_1
B U	L	120 CCW	2 3	8 6	75	>1 000	0 266
		120 CW	1 7	11 9	54	>1000	0 147
D V	R	120 CCW	8 8	11 5	213	530	0 758
		120 CW	8 3	12 5	308	750	0 666
F A	R	90 CCW	5 2	10 0	180	480	0 522
		90 CW	6 2	16 2	121	183	0 377
F Q	R	120 CCW	3 8	8 3	120	900	0 458
		120 CW	5 3	8 4	104	750	0 633
M G	R	150 CCW	5 1	14 5	200	595	0 353
		150 CW	5 9	9 2	250	950	0 640
M A	L	120 CCW	2 1	14 2	134	800	0 150
		120 CW	1 4	10 0	180	900	0 142
L C	R	120 CCW	3 7	27 3	56	>1 000	0 133
		120 CW	3 6	19 7	146	>1 000	0 183
P A	L	120 CCW	15 9	25 4	187	>1 000	0 625
		120 CW	6 9	25 4	81	770	0 275
S C	R	120 CCW	2 3	8 2	296	950	0 283
		120 CW	2 8	5 1	120	900	0 550
V C	R	120 CCW	4 7	20 4	358	>1 000	0 233
		120 CW	8 3	13 2	112	>1 000	0 625



3 Values of the gain K and the long time constant of the semicircular canals for normal subjects (o) and ...iere's patients (•)

Even when all subjects were examined dur... periods free from vertiginous attacks, a ...eat variability was observed in their nystag...ic responses According to Stahle's observa...ons (1968), spontaneous or positional nystag...us was present in few cases Its direction ...as toward as well as away from the diseased ...r

The results obtained from 10 Meniere's pa...ents are summarized in Tables I and II ...able I reports clinical data, the amplitude and ...rection of the post-rotational stimulation, ...nd the values of some nystagmus parameters ...umber of beats, duration of the first phase, ...nd peak slow phase velocity) Table II gives ...e estimated value of the model parameters $T_1 T_2 T_3$, and the ratio K/T_1 The most out...anding result emerging from Table II is the ...w values of the parameters K and T_1 Fig 3 ...ves the values of K and T_1 for normal sub...cts and Meniere's patients the separation ...tween the two sets of data is evident

As for the balance between CW and CCW ...sponses in Meniere s patients, a compara...ve analysis suggest that K is smaller in re...ponses recorded after turning toward the in...ct side, while no systematic difference exists ...r T_1 This observation is supported by the ...resentation of data as in Fig 4, where the ...tio K_D/K_I and T_{1D}/T_{1I} are reported Subs... D and I refer to responses induced by utriculopetal cupula deflections in the diseased and the intact semicircular canal, respectively (that is, post-rotational responses obtained after turning toward the intact and the diseased side) If the two responses were perfectly balanced with no predominance in one particular direction, all the data would fall into a small region centred around the point (●1, 1) Since the majority of data are clustered into the lower quadrants, without any significant difference between the right and the left one, it can be concluded that an asymmetry exists only in the sense previously outlined

When the ratio K/T_1 is computed from CW and CCW responses (see Table II), smaller values are obtained for the diseased side Simple manipulation of eq (1) prove that the ratio K/T_1 is proportional to the predicted peak slow phase velocity V_p of post-rotational responses ($V_p = AK/T_1$, where A is chair velocity before stop) Thus the ratio K/T_1 provides the same information as the *maximum intensity* defined by Stahle (1968) for caloric tests, and it is equally sensitive to variations in labyrinthine excitability

The ratio K_D/T_{1D} is related to the hearing loss in the diseased ear The results are given in Fig 5 they prove a strict correlation be-

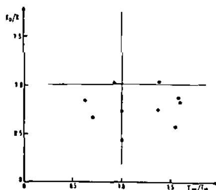


Fig 4 Balance between the diseased and intact side in terms of K and T_1 Subscripts D and I refer to responses induced by utriculopetal cupula deflections in the diseased and the intact semicircular canal respectively (th... is post rotational responses obtained after turning

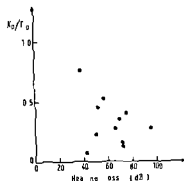


Fig 5 Relationship between the ratio K/T_1 and the hear
... The reported
the fre

tween these two factors in the sense previously reported by Enander & Stahle (1967) for maximum intensity and mean auditory threshold

In all the examined patients caloric responses proved an hypoexcitability of the diseased labyrinth and an almost normal excitability of the intact one

DISCUSSION

While caloric tests point out a marked asymmetry in the nystagmic response of unilateral Meniere's patients, the same feature does not appear so clearly in post rotational tests The results reported in this paper show that the parameters K and T_1 are only slightly different in CW and CCW responses On the other hand, they are both significantly reduced with respect to normal values The results are in good agreement with previous findings by Wilmot (1974)

Since the time constant of the intact semicircular canal cannot be varied, its apparent decrease can only be explained as the effect of a process of central compensation taking place through a reduction of the intact side function

Similar processes have been observed in vestibular compensation following hemilabyrinthectomy in the cat (Precht, 1974 Courjon et al , 1977) In the acute stage, post rotationa stimulations of the injured side do not produce a reversal of spontaneous nystagmus In the compensated stage, spontaneous nystagmus has disappeared, and a high level of symmetry is attained Nevertheless, both CW and CCW responses are reduced with respect to norma ones

The results of electrophysiological investigations suggest that this functional recover occurs through the following distinct mech anisms an inhibitory control exerted by vestibulo-cerebellum upon the vestibu nuclei of the intact side, a recalibration of th gains of the commissural pathways betwee the two sets of vestibular nuclei, and a generation of resting activity in the dea ferented vestibular nuclei (Precht et al , 19 McCabe et al , 1972)

Such a process has been modelled by a suming a two-level hierarchical organizati the vestibulo ocular system (Jeannerod Schmid, 1977) the first level was represent by the vestibulo ocular reflex, while the se ond one was localized in the cerebellum W a strong imbalance between the two vestibu receptors occurs, the second level sets u strategy of control of the lower level aimed

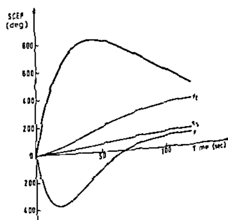


Fig 6 *Upper diagram* time course of the slow cu tive eye position (SCEP) for a step change in head a velocity *Lower diagrams* time course of the sens coefficients v_i of the SCEP with respect to the tim stants T_1 T_2 and T_3

he re establishment of the static (absence of spontaneous nystagmus) and dynamic balance symmetry of the responses)

The results reported in this paper suggest that a similar strategy takes place in unilateral Meniere s disease Since the second level operates through inhibition of the first level the symmetry of the post rotational responses can be achieved in Meniere s patients only through a depression of the intact side Thus in the compensated stage both CW and CCW responses of Meniere s patients are reduced with respect to normal subjects The lower symmetry observed in caloric responses with respect to post rotational responses can also be explained In fact in the former case due to the unilateral nature of the caloric stimulation the contribution to the system balance given by recalibration of the gain of the commissural pathways is lost

Surprisingly the data given in Table II indicate no systematic variations of the mechano-neural transduction time constant T_2 between the intact and the diseased side Moreover in spite of the great variability of T_2 in Meniere s patients the observed values can still be considered within the range of normality This result suggests that at the time of examination no pathological changes of the labyrinthine epithelium such as to produce appreciable variations in the dynamics of the mechano-neural transduction process were present It is possible that only the gain of this process was attained The reduction of the gain of the overall reflex seems to support this hypothesis

CONCLUSION

The results reported in this paper seem to revalue the rotatory tests in the examination of Meniere s patients Mainly for what concerns the evaluation of the dynamic balance between the two vestibular pathways rotatory tests should be preferred to caloric tests Actually vestibular stimulations in everyday life are always bilateral Thus it is likely that the fine control mechanisms developed to maintain or reestablish the balance between the right and the left vestibular pathways are not recruited in the case of unphysiological unilateral stimulation The persistent asymmetry of the caloric response observed also in longstanding Meniere s patients seems to support this hypothesis On the contrary the comparison of the values of the vestibulo-ocular reflex parameters estimated from CW and CCW post rotational responses indicate the possibility of quantifying the level of compensation It is also proved that this central compensation is obtained through a depression of the vestibular function of the intact side

APPENDIX

The sensitivity of dynamic systems to parameter variations is currently considered in control theory by introducing the *sensitivity coefficients* s_i defined as

$$s_i(t)=\frac{\delta y(t)}{\delta \ln p_i}$$

where $y(t)$ is the system output and p_i is the ith system parameter (Tomovic 1963) Sensitivity theory can also be used to evaluate the reliability of a parameter estimation from recorded responses Actually the greater the sensitivity coefficient of one parameter is the more accurate its identification results From this point of view it is of interest to estimate the sensitivity of the slow cumulative eye position (SCEP) to variations of the parameters T_1 T_2 and T_3 characterizing the dynamics of the vestibulo-ocular reflex We can thus introduce the following sensitivity coefficients

$$s_1(t)=\frac{\delta c(t)}{\delta \ln T_1} \quad s_2(t)=\frac{\delta c(t)}{\delta \ln T_2} \quad \text{and } s_3(t)=\frac{\delta c(t)}{\delta \ln T_3}$$

where $c(t)$ is the time function representing the SCEP

Fig 6 shows the diagram of the SCEP predicted by eq (1) for a step change in the angular velocity The following mean val

timated on a population of 25 normal subjects were given to model parameters: $K=10$, $T_1=20$ sec, $T_2=150$ sec, and $T_3=500$ sec. In the same figure, the sensitivity coefficients $v_1(t)$, $v_2(t)$, and $v_3(t)$ are presented. It is clear that the dynamics of the primary phase of post-rotational response is dominated by the long-time constant T_1 of the semicircular canals. The sensitivity of the response to variations of T_2 becomes significant at the end of the primary phase and increases further during the secondary phase. The sensitivity coefficient v_3 is always small. Therefore, the parameter T_3 can only be crudely estimated.

RÉSUMÉ

Les principaux paramètres du reflexe vestibulo-oculomoteur (le gain ou reflectivité, la constante de temps majeure des canaux semicirculaires, la constante de temps de la transduction mécano neurale et celle de l intégrateur centrale) ont été calculés à partir des réponses nystagmiques post rotatoires pour des sujets souffrant de maladie de Meniere unilaterale La comparison avec les mêmes resultats obtenus chez des sujets normaux montre une diminution des deux premiers paramètres soit dans les réponses horaires que dans les réponses anti horaires Au contraire les autres paramètres ne mon trent pas des differences significatives Vraisemblablement à la suite d'une phenomène de compensation centrale, un niveau éleve de symétrie a été observé chez sujets avec un maladie de Meniere datante de plus es annees

ZUSAMMENFASSUNG

Die wichtigsten Parameter des vestibulo-okularen Reflexbogens (d h der Verstarkungsfaktor, die lange Zeitkon stante der Bogengänge, die Zeitkonstante der peripheren Adaptation und die des zentralen „leaky integrator") wurden aus dem postrotatorischen Nystagmus von Patienten berechnet, die an einem unilateralen M Meniere litten Der Vergleich mit normalen Versuchspersonen zeigte eine Abnahme der ersten beiden Parameter sowohl fur Drehungen im als auch entgegen dem Uhrzeigersinn Dagegen ergaben die ubrigen Parameter keine signifikanten Änderungen Vorwiegend bei Patienten mit langerer Anamnese wurde eine großere Symmetrie be obachtet, dies ist vermutlich durch eine zentrale Kompen sation zu erklaren

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COMPUTER CALCULATION OF MOVEMENT OF BODY'S CENTER OF GRAVITY

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Abstract The movement of the body s center of gravity was calculated in normal subjects and in vertiginous patients by using a strain gauge platform system and a digital computer The total length of the loci traced by the center of gravity in 40 normal subjects during normal standing for 1 minute was 52 0±18 5 cm with eyes open and 81 5±52 3 cm with eyes closed The ratio of the total length with the eyes closed compared with that with the eyes open during normal standing for 1 minute was 1 56±0 56 The time course of the length of the locus was calculated every 10 seconds and characteristic curves were obtained The dependability of this test appeared to be fairly accurate and the usefulness of this technique for the diagnosis of vertiginous or ataxic diseases is recommended

The righting reflex, characterized by Rom-…'s test, has been an important tool in neu…ogical diagnosis However, there is no exact criterion for judging the results of the righting reflex How wide a margin should be allowed between normal and ataxic subjects? Are there any characteristics of body sway particular to the standing human suffering from vertigo? These are matters of concern to those conducting research on the body's center of gravity, which is one of the indicators by which the degree of instability is expressed

Recently, recording of the displacement of the body's center of gravity by means of the stabilograph has been introduced This method of calculating the displacement consists of measuring on paper the area of the printed trace obtained from the center of gravity The most common defect of this method is that errors are liable to show up in the results caused by accidental deflection occurring …ing the recording of the body's displacem… such as can arise when the body sway, w… standing, is disturbed by a lurch caused by… subject's lack of concentration The calcul… area may then be too great in comparison … the possible minimal displacement

The present study was carried out … computer assistance, with the aim of obtai… exact data on the movement of the bo… center of gravity

MATERIAL AND METHOD

The Static Sensonograph constructed by Sanei Co … kyo Japan using the strain gauge technique was use… a subject standing on the platform in order to obt… continuous record of the extent of movement of the… (CG is the abbreviation of body s center of gravity … the horizontal plane The output from the sensonogr… in terms of voltage was connected to a plotting unit … at the same time suppl ed … data of l… magnetic … into a d… o Tokyo … further analysis (Fig 1)

The program for operation of the computer was wr… by Assembly Language and the object program for c… lation of the locus was applied The sampling time … 0 125 sec and the analysis time was one or two min… The calculated results were printed out in digital v… every 10 sec

The study concerned 40 healthy test subjects (20 m… and 20 females) aged 16 to 30 Five subjects with ve… underwent the same test Each subject was placed o… platform with the feet together and was asked to … … the following … d of tim… ht ahea… ght tow…

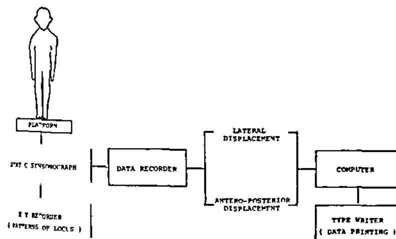


Fig 1 Block diagram of equipment

downward and upward) 3) Mann s position with right foot forward or the left foot forward and 4) standing on a 15° tilted platform (rightward leftward forward and backward)

RESULTS

Total length of locus traced by CG during standing

Table I shows the means and the upper rejection limits of the normal range in the total length of the locus traced by the CG during the first minute of standing The difference in locus length obtained when standing with eyes open vis a vis with eyes closed was statistically significant in all conditions tested ($p<0.05$) In the different head positions with eyes open, the means with head inclined downward and upward were greater than with the other head positions ($p<0.05$) However in the condition with eyes closed no differences among the values of the five head positions were revealed ($p>0.05$) In the data obtained by the Mann's positions, no statistical difference was found between the values with the right foot forward and those with the left foot forward ($p>0.05$) There were no differences between the values on the platform tilted in the four different planes ($p>0.05$)

Ratio of total length of locus traced by CG with eyes closed compared with that with eyes open (EC/EO)

The gap in the length of the locus, traced by the CG with eyes open and that with eyes closed, would be considered to indicate the grade of optical compensation of the righting reflex in the body s stabilizing system The

Table I *Total length of locus traced by CG during 1 minute of standing ($N=40$)*

	Head positions					Mann s positions		Platform 15° tilted			
		Head inclined									
	Straight	Towards the right	Towards the left	Down ward	Up-ward	Right foot forward	Left foot forward	Right ward	Left ward	For ward	Back ward
Means											
Eyes open	52 0	54 4	55 4	61 2	61 2	84 6	86 3	71 6	66 4	61 7	65 1
Eyes closed	81 5	79 5	78 4	78 8	88 0	161 4	159 9	104 7	106 9	98 5	100 9
Upper rejection limits ($p=0.05$)											
Eyes open	70 5	73 9	71 7	87 0	85 0	121 0	122 4	100 3	94 8	88 8	93 8
Eyes closed	133 8	133 4	121 0	123 6	139 1	225 4	226 0	[illegible]	[illegible]	[illegible]	[illegible]

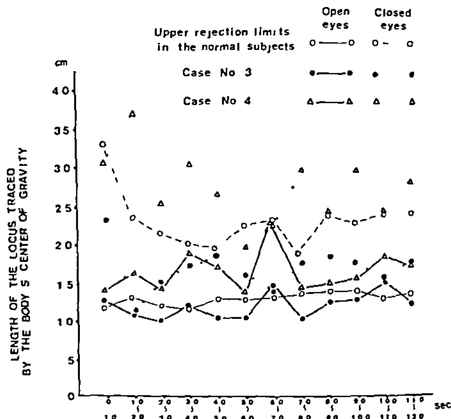


Fig 2 Time course of th length of locus traced by (The t me course of Case I 3 with orthostat c hypoter sion and that of Case No with acoustic neuroma w shown with the upper rej tion limits in the normal s jects (N=3? p=0 0?)

smallest ratio (EC/EO) was obtained when the head was inclined downward while the largest shown in Mann's position with the right forward (Table II)

Time course of length of locus traced by CG

The length of the CG locus in the straight head position for 2 min was calculated every 10 sec (Fig 2) The time course of the length with eyes open was very stable However a characteristic curve was obtained in the trials with eyes closed, showing an initial large (flection followed by several small deflectior

Test–retest reliability

The length of the locus traced by the CG in straight head position during the first one i of standing was measured twice with an terval of one week on 32 subjects The sults calculated were 52 0±18 5 cm 50 3±18 0 cm There was no statistic significant difference between them (p>0 (

Table II *Ratio of the total length of locus traced by CG with eyes closed compared with with eyes open means ± rejection limits (N=40)*

Head positions										
	Head inclined				Mann s positions		Platform 15° tilted			
Straight	Towards the right	Towards the left	Down ward	Up ward	Right foot forward	Left foot forward	Right ward	Left ward	For ward	Back ward
1 56±0 56	1 47±0 52	1 44±0 50	1 31+0 50	1 50+0 63	1 94+0 8?	1 88±0 68	1 47±0 66	1 6?±0 69	1 57±0 68	1 43+

Table III *Total length of locus traced by CG (L) and ratios of the total length with eyes closed compared with that with eyes open (EC/EO) in the vertiginous patients during 1 minute of standing*

A and N denote abnormal and normal values respectively as compared with the upper rejection limits in the normal subjects

	Head positions					Mann s positions					
		Head inclined						Platform 15° tilted			
	Straight	Towards the right	Towards the left	Down ward	Up-ward	Right foot forward	Left foot forward	Right ward	Left ward	For ward	Up-ward
Case no 1											
L eyes open	A	A	A	A	A	A	A	A	A	A	A
L eyes closed	A	A	A	A	A	A	A	A	A	A	A
EC/EO	A	A	A	N	A	N	N	N	N	N	A
Case no 2											
L eyes open	N	N	N	A	A	A	A	N	N	N	A
L eyes closed	A	N	N	A	A	A	A	N	A	A	A
EC/EO	N	N	N	N	N	A	A	N	N	A	A
Case no 3											
L eyes open	N	N	A	A	A	N	N	A	A	A	A
L eyes closed	N	N	A	A	A	N	A	N	N	A	N
EC/EO	N	N	N	N	N	N	N	N	N	N	N
Case no 4											
L eyes open	A	A	A	A	A	☆	☆	☆	☆	☆	☆
L eyes closed	A	A	A	A	A	☆	☆	☆	☆	☆	☆
EC/EO	N	A	N	A	A						
Case no 5											
L eyes open	A	A	A	A	A	☆	☆	☆	☆	☆	☆
L eyes closed	A	A	A	A	A	☆	☆	☆	☆	☆	☆
EC/EO	A	N	A	A	N						

☆ Not measurable

The practice of calculating the CG movement

To illustrate the clinical value of this method the results of 5 patients are described here (Table III)

Case No 1 (62 year old female) She has been suffering from vertigo after sudden deafness in the left ear Romberg s test and Mann s test were positive only with eyes closed However, the calculated length of the locus of the CG was above the normal limit under the conditions with eyes open and with them closed

Case No 2 (23 year old female) A brain stem lesion was considered to be the cause of her instability Abnormal results were obtained especially in the Mann s positions

Case No 3 (22 year old female) She had the typical symptoms of orthostatic hypotension Romberg s test and Mann s test were negative However pathologic results were obtained with the computer

Case No 4 (56-year-old female) Acoustic neuroma in the left cerebello-pontine angle was suspected on the basis of otoneurologic tests and X ray examination Recently the diagnosis was confirmed at surgical operation She was too unstable to undergo the tests in Mann s positions and on the tilting platform

Case No 5 (36-year old male) He was in the active stage of Ménière s disease Instability made it impossible for him to stand Mann s test or the tilting platform test

Table III shows the ratio of EC/EO of the above mentioned 5 patients All cases except No 3 showed abnormal values Cases 1 and 5, with left sided peripheral vestibular lesions, had large values when the head was inclined towards the left

The time course of the CG deflection was almost normal in the functional disease, such as in Case 3, while Case 4, with an organic lesion, presented a greatly fluctuating and irregular curve (Fig 2)

DISCUSSION

So far, many investigators have been engaged in quantitative measurement of the CG (Hellebrandt, 1938, Hellebrandt & Braun, 1939, Travis, 1944, 1945, Wapner & Witkin, 1950, Baron, 1964, Begbie, 1967, Baron et al , 1968, Njiokiktjien & Folkerts, 1971, Kapteyn & deWit, 1972, Benson & Jobson, 1973, Taguchi & Yoda, 1975)

In 1938 Hellebrandt estimated the maximal shifts in the center of gravity from kymographically recorded changes in a balance weight, which occurred when a subject stood on the [illegible] t of series of platforms constructed [illegible] two scales concurrently (of du Boismond) placed at right angles to each other [illegible] vis (1944) used a platform mounted on a universal joint Wapner & Witkin (1950) used a stabilometer made of a wooden table upon which a subject stood while trying to keep it horizontal Thomas & Whitney (1959) attempted to describe the postural sway in a more objective and mechanical manner, using a three dimensional platform equipped with a strain gauge system Since then the strain gauge platform system has prevailed for recording the movement of the CG (Henriksson et al , 1966, Kapteyn & deWit 1972 Benson & Jobson, 1973, Taguchi, 1974) Begbie (1967) measured the postural sway by two potentiometers fixed to the pivots which supported the platform Another device was used by Baron et al (1968) and Njiokiktjien & Folkerts (1971) They recorded the body motion by using a four point square detector plate transforming variations of pressure int electric information by means of electr magnetic plungers

The present research aims to calculate th exact value of the locus traced by the CG s that mutual comparison of the data betwee different stages in the disease of a patient c between normal people and patients sufferin from vertiginous or ataxic diseases can b made

In this study the amplification of our ap paratus was adjusted so that the 100% produ of body weight and body length of each te subject on the platform would give the sam deviation of the tracing of the CG, in order t simplify the calculation of the results Th same device was adopted by Henriksson et a (1966)

Our statistical analysis indicates that th length of the locus traced by the CG during minute of standing showed a greater or less difference according to the head inclination the foot positions and the platform tilt Ve tiginous or ataxic patients showed longer lo than did normal subjects

EC/EO implies the grade of visual compe sation for body balance (Wapner & Witki 1950, Kapteyn & deWit, 1972) In our data th EC/EO of 4 vertiginous patients was outsi the rejection limits for the normal subject Using this indicator, it will be possible to d ferentiate vertiginous or ataxic diseases una fected by eye closing such as orthostat hypotension and cerebellar ataxia

The time course of the CG revealed t characteristic deflection in the length of t locus during standing with eyes closed T initially large deflection and the subseque several small deflections would be explicab by Nashner's model When the ankle joint r flexes are active, postural stability will b achieved in preventing the body from diver ing, resulting in an increase in depth sensatio exteroceptive and vestibular cues (Nashn 1970)

The test–retest values showed that the c

'lity of this test was adequate for clini- These normal and clinical results sug- that the measurement of the CG move- t seems to offer the possibility of being oped into a new diagnostic technique for erliginous and ataxic patients

ZUSAMMENFASSUNG

~ Bewegung des Korperschwerpunktes wurde an nor pathologischen Versuchspersonen mittels eines a osensonographs und einer elektronischen Rechenma berechnet Die totale Lange des vom Körper schwerpunkt geschriebenen geometrischen Ortes an nor Personen bei Stehen fur eine Minute war 52 0±18 5 cm mit offenen und 81,5±52 3 cm mit geschlossenen Augen Die Rate der totalen Lange mit geschlossenen Augen zur derjenigen mit offenen Augen an normalen Per sonen bei Stehen für eine Minute war 1 56±0,56 Der Ver f der Bewegung des Korperschwerpunktes wurde jede ehn Sekunden berechnet und die characteristische Kur e wurde erzielt Die Verlassenheit und die Gultigkeit von lessen der Korperschwerpunktbewegung in klinischen tuationen wurde veranschaulicht

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SYMPATHETIC NERVES AND NASAL SECRETION IN THE CAT

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Abstract The influence of sympathetic nerves on nasal secretion was examined in anaesthetized cats The results from experiments in which the Vidian nerve was stimulated supramaximally for 3 min show that vasoconstriction occurs in the nasal cavity during the production of nasal secretion The amount of nasal secretion produced in the normal cavity on Vidian nerve stimulation was greater than that produced in the sympathectomized nasal cavity but only significantly so at 2 and 5 Hz It is concluded that at these lower frequencies sympathetic activity induced by Vidian nerve stimulation increases nasal secretion

The Vidian nerve conveys preganglionic parasympathetic secretory, vasodilator and postganglionic sympathetic vasoconstrictor nerves [illegible] the nasal cavity of the cat (Malcomson [illegible]9, Eccles & Wilson, 1973, Malm, 1973, [illegible], 1974) Supramaximal stimulation of the facial nerve in the brain stem evokes secretion in the nasal cavity (Eccles & Wilson, 1973) Similar amounts of nasal secretion were also produced by supramaximal stimulation of the Vidian nerve (Eccles & Wilson, 1973) although these stimulation parameters evoke vasoconstriction in the nose (Eccles & Wilson, 1974) In these studies secretion was measured after 3 min stimulation and vascular responses recorded only during 15 sec stimulation

The present investigations were carried out on anaesthetized cats in order to examine the role of sympathetic nerves in nasal secretion In preliminary experiments the Vidian nerve was stimulated at vasoconstrictor and vasodilator parameters (Eccles & Wilson 1974) to determine the extent and duration of each type of vascular response The amount of secretio evoked at both these parameters was al examined In further experiments the secr tion produced from the normal cavity on V dian nerve stimulation was compared with th evoked from the sympathectomized nas cavity

Some of these findings have already be demonstrated to the Physiological Socie (Wilson & Yates, 1976)

METHOD

Cats of either sex, free from nasal infecti and 2–4 kg body weight were anaesthetiz with pentobarbitone sodium 40 mg/kg i p a the trachea cannulated

Vascular changes in the nose were record as pressure changes in the sealed nasal cav by means of a short plastic cannula wh was inserted into the nostril and connected a pressure transducer (Bell & Howell ty 4 327 223) and a Devices M2 pen recor (Wilson & Yates, 1975) This method reco only the overall vascular changes in the n and mainly reflects the changes in the cap tance vessels (Anggård & Edwall 1974 Ma 1974) Nasal secretion was collected from nasal cavity by placing in the nostril a cann connected to a pre weighed bottle to wh continuous suction was applied (Eccles Wilson, 1973) The recording of vascular sponses and collection of nasal secretion w undertaken in separate experiments

The Vidian nerve was exposed in the o

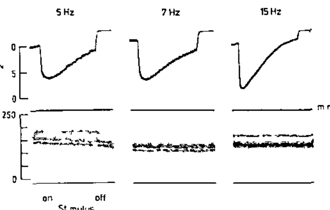


Fig 1 Vasconstrictor responses evoked on supramaximal 3 min Vidian nerve stimulation Lower record shows arterial blood pressure

the method of Eccles & Wilson (1973) : cut peripheral end was placed on bipolar inum electrodes and covered with a small on wool swab soaked in liquid paraffin ᐩ) The sphenopalatine and infraorbital ves and cervical sympathetic chains were ioned The Vidian nerve was also exposed some cats in which the superior cervical glion had been removed 10–14 days pre usly and thus the Vidian nerve was purely asympathetic

quare wave impulses were delivered from rass S4 stimulator through an isolation unit U4) In secretion experiments the Vidian ve was stimulated supramaximally (7 0 V) ng a pulse width of 1 0 msec and at 2 0 V 1 0 2 msec The nerve was stimulated for in and allowed to rest for 7 min, the secre- n bein collected over a 10-min period The e of flow of secretion was expressed as mg/ nin

In experiments in which vascular responses re recorded the Vidian nerve was stimu ed at the same parameters for 3 min every min

In all experiments the arterial blood pres re was recorded from a femoral artery by eans of a pressure transducer (Bell & Howell pe 4 327 221) and a pen recorder (Devices 2)

Non paired comparisons and Student s *t*- st were used in the statistical analyses

RESULTS

Duration of vasoconstriction and vasodilation produced in nasal cavity by Vidian nerve stimulation

(*a*) *Vasoconstriction* Previous studies on Vidian nerve induced nasal secretion in the cat have shown that secretion appears be tween 2 and 5 Hz, increases with increasing stimulation frequencies, to reach a maximum value between 10 and 15 Hz (Eccles & Wilson, 1973)

The cut peripheral end of the Vidian nerve was therefore stimulated supramaximally in 4 cats for 3 min at 5, 7 and 15 Hz The cats were given atropine sulphate (50 μg/kg i v) to prevent a pressure change in the nasal cav ity caused by accumulation of nasal secretion which could interfere with the recording of vasoconstriction

The results obtained from one cat (which are typical of those in the remaining three) are shown in Fig 1 At all frequencies, vasocon striction appeared immediately on stimulation to reach a maximum value at approximately 10 sec but the extent to which it occurred and the duration for which it was sustained de pended on the stimulation frequency Thus at 5 and 7 Hz it fell to between 60 and 70 initial value by 90 sec and to 60% at 3 min At 15 Hz clined rapidly reaching 30 to

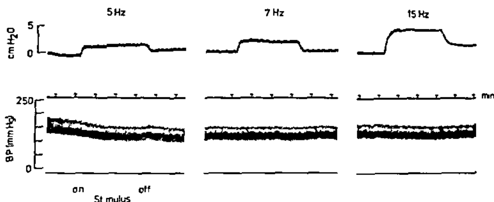


Fig 2 Vasodilator responses evoked on 3 min Vidian nerve stimulation at 2 0 V 0 2 msec Lower record shows arterial blood pressure

response by 90 sec and it was undetectable after 3 min

(*b*) *Vasodilation* Vasodilation was evoked at 5, 7 and 15 Hz in the nasal cavity of 4 cats by stimulating the Vidian nerve with parameters of 2 0 V and 0 2 msec Parameters greater than this could not be used as they produce vasoconstriction

Vasodilation appeared more slowly than vasoconstriction and increased with increasing stimulation frequency In contrast to the vasonstrictor responses, vasodilation remained stant throughout the 3 min stimulation riod, returning slowly to the pre stimulation level only after stimulation had ceased The findings in one of these experiments are shown in Fig 2

Secretion studies

(*a*) *Secretion induced by parameters which induced vasoconstriction and vasodilation*

Nasal secretion was collected from each of 6 cats by stimulating the Vidian nerve at each of the vasoconstriction and vasodilator parameters at frequencies ranging from 2 to 25 Hz

Using vasoconstrictor parameters watery nasal secretion appeared 30 sec after stimulation began The minimal effective frequency was 2–5 Hz, the flow increasing with increased stimulation frequency to reach a maximum value of 10–20 Hz The maximum rate of flow of secretion in 6 cats ranged from 4[illegible] to 114 mg/3 min (mean 83 4±11 4 S E)

In these 6 cats, nasal secretion could only be evoked in 3 when using vasodilator parameters When secretion did occur, it appeared after 30 sec between 2 and 5 Hz to reach a maximum value between 15 and 20 Hz At each frequency the flow rates were only approximately 80% of those obtained with vasoconstrictor parameters

(*b*) *Secretion in sympathectomized nasal cavity*

To investigate further the role of sympathetic nerves on the secretory activity of the nasal mucosa, nasal secretion evoked in 6 normal cats was compared with that obtained from the sympathectomized cavity of 6 cats by supramaximal Vidian nerve stimulation The stimulation parameters only produced vasodilation in the denervated cavity

A watery nasal secretion appeared after an interval of 30 sec at a frequency of 2 Hz from both the normal and the sympathectomized nasal cavity In both instances the secretion increased with increasing stimulation frequency, to reach a maximum value at 15 Hz These findings are shown in Fig 3 In order to eliminate the variation in body weight, secretion was expressed as mg/3 min/kg

The mean values for secretion obtained

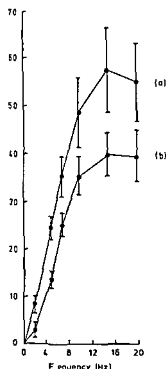


Fig 3 Secretion frequency/response curves obtained in the normal cavity (b) the sympathetically denervated ty on supramaximal 3 min Vidian nerve stimulation Each value is the mean of those obtained in 6 cats (S E bars included)

from the normal cavity were greater than those from the sympathectomized cavity at all frequencies—but only significantly greater at 2 and 5 Hz ($P<0\ 05$)

DISCUSSION

Previous studies have shown (Eccles & Wilson 1973 1974) that stimulation of either the facial nerve in the brain stem of the cat, or the Vidian nerve, evokes similar amounts of nasal secretion Whereas brain stem stimulation causes vasodilation in the nasal cavity, Vidian nerve stimulation induces vasoconstriction These findings suggested that the accompanying vasoconstriction has little effect on the secretory activity of the nasal mucosa The main criticism is however that secretion was measured for 3 min, whereas the vascular responses were produced only by 15 sec stimulation

When, however, the Vidian nerve was stimulated for 3 min at frequencies when secretion appears (5 Hz), when it is increasing (7 Hz) and reaches a maximum (15 Hz), vasoconstriction reaches its peak value within seconds Vasoconstriction then declines in a frequency-dependent manner during the 3 min stimulation period At all frequencies it is still present after 90 sec and only at 15 Hz was it undetectable after 3 min Since nasal secretion appears in the cannula after 30 sec, vasoconstriction must be present during the secretory activity of the nasal glands

In contrast, vasodilation evoked by weaker stimuli was sustained throughout the 3 min stimulation period Maximum vasodilation could not be elicited because in crease of stimulus intensity produces vasoconstriction The threshold of vasodilator fibres was apparently lower than that of secretory fibres because secretion occurred in only 50% of the cats using vasodilator parameters

Supramaximal stimulation of the Vidian nerve to the sympathectomized cavity evoked only vasodilation Nasal secretion appeared after the same latent period as in the normal cavity, indicating that the immediate vasoconstriction occurring in the normal cavity had no effect on the initial production of nasal secretion The secretion/frequency response curves obtained from the normal and sympathectomized cavities were also similar Although the mean values of secretion from the normal cavity were greater than those from the sympathectomized cavity at all frequencies, they were only significantly greater at 2 and 5 Hz, suggesting that at these frequencies sympathetic activity may aid the production of secretion

These findings are in contrast to the histological studies of Dahlstrom & Fuxe (196ᶜ who could not detect adrenergic fibres the glands of the nasal mucosa of the guinea pig and rabbit although A

Densert (1974) reported sparse adrenergic innervation of the nasal mucosal glands of the cat.

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ZUSAMMENFASSUNG

Der Einfluß von sympathischen Nerven auf die Nasensekretion wurde an narkotisierten Katzen untersucht. Supramaximale Reizung des Nervus vidianus fur drei Minuten ergab ein Nasensekret und eine gleichzeitige Vasokonstriktion in den Blutgefäßen der Nasenhohle. Die durch Vidianusreizung ausgelöste sezernierte Menge von Nasensekret in der normalen Nasenhohle war großer als die, die bei diesen Reizfrequenzen in der sympathektomisierten Nasenhohle gemessen wurde, aber nur bei 2 und 5 Hz war der Unterschied signifikant. Daraus kann man schließen, daß bei diesen niedrigen Reizfrequenzen, die durch Reizung des Nervus vidianus hervorgerufene Nasensekretion von der gleichzeitigen Stimulation der in diesem Nerven verlaufenden sympathischen Fasern gesteigert wird

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THE DOMESTIC PIG AS AN EXPERIMENTAL ANIMAL FOR STUDIES ON THE NASAL CYCLE

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Abstract The resistance to air flow of each nasal passage in the anaesthetized pig was determined by pumping air through the nose and measuring pressure changes in nasal cannulae A nasal cycle was observed in the majority of the pigs Three types of change in nasal resistance were observed and their possible causes are discussed Section of the right cervical sympathetic nerve abolished the cyclic changes in nasal resistance of both nasal passages The method described may prove useful in studying the physiology of the nasal cycle

The phenomenon of the nasal cycle is well established in man (Heetderks, 1927, Stoksted 1953) and more recently cyclic changes in nasal resistance have been demonstrated in laboratory animals (Bojsen Møller & Fahrenkrug 1971, Eccles & Maynard, 1975) The nasal resistance is determined by the state of engorgement of the erectile tissue in the nasal mucosa and this is controlled by the autonomic nervous system There is some evidence that the nasal cycle is regulated through the sympathetic innervation of the nose (Beickert, 1950 Stoksted & Thomsen 1953) and this is based on studies involving local anaesthesia of the stellate ganglion in man Since the period of the nasal cycle is between 0 5–4 hours (Heetderks, 1927) and the duration of the local anaesthesia is only about 2 hours it is difficult to interpret whether or not the procedure has abolished the nasal cycle In the present study the pig has been used as an experimental model to study the role of the sympathetic innervation in the regulation of nasal resistance The anatomy of the nasal cavity and the structure of the nasal mucosa of the pig are very similar to man (Melon 1968) and the methods used in the present study may prove useful in future studies to determine the function of the nasal cycle in man

METHODS

Thirteen 'Large White' pigs were used for the experiments (6 female, 6 male, 1 castrate male, body weights 16–50 kg) The pigs were anaesthetized with 'Halothane' through a face mask, then the trachea was intubated and the pigs maintained on closed circuit Halothane anaesthesia Each pig was anaesthetized for a 4-5-hour period at intervals of 7–14 days In every experiment nasal resistance was recorded during the period 10 00–16 00 hours The pigs were rested on a thick foam rubber mat to prevent pressure sores and then covered with an electric blanket to maintain the body temperature at 39°C The range of laboratory temperature throughout the period of the experiments was 18–22°C with a range of relative humidity of 25–50% saturated

The resistance to air flow of each nasal passage was determined by means of a twin cylinder pump which moved air through the nasal passages as shown in Fig 1 The posterior nares were cannulated with a 10 mm internal diameter rubber tube with a shaped copper end piece to ensure a free flow of air from the nose to the mouth Specially designed plastic cannulae were fixed to the nostrils by means of a slight vacuum over the skin surrounding the nostril and an airtight seal was easily made on the smooth surface of the pig's nose The pump was driven at 29 r p m by means of

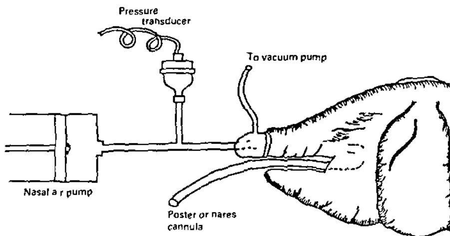


Fig 1 Diagram of determining nasal resistan
showing the position of th
pump and cannulae

electric motor and a volume of 84 ml was moved by each piston stroke Pressure transducers of the type used to monitor arterial blood pressure (Consolidated Electronics type 4 327 L221) were connected to side arms from each nasal cannula (see Fig 1), and the pressure signals were displayed on a 'Devices' four-channel pen recorder The change in pressure in the nasal cannula with each stroke of the pump was directly related to the resistance to air flow of the nasal passage The pressure transducers were calibrated with a water manometer at the start and end of each experiment and the two cylinders of the pump and the nasal cannulae were regularly checked with standard resistances to ensure that each half of the pump and recording system was identical

The respiratory rate, heart rate and body temperature were recorded at 30 min intervals throughout the experiment The respiration was also continuously monitored by means of a mercury in rubber strain gauge strapped around the chest of the pig The signal from the strain gauge was displayed on the pen recorder trace

Section of the cervical sympathetic nerve

The right cervical sympathetic nerve was sectioned under Halothane anaesthesia The nerve was identified by monitoring nasal resistance (as above) and noting the effects of clamping, section, and electrical stimulation of the cut peripheral end of the nerve These proce
dures should cause nasal vasoconstriction an
a decrease in the resistance of the ipsilater
nasal passage if the sympathetic fibres to th
nose are present in the nerve

RESULTS

The method described for determining nasa
resistance has proved successful and has bee
developed over a period of 3 years of experi
mentation with different techniques The ma
jor problems encountered in the developmen
of the method have been the cannulation of th
nostrils and the maintenance of a free airwa
from the posterior nares to the mouth Thes
problems have been overcome by the develop
ment of special suction cannulae to attach t
the nostrils and a specially designed posterio
nares cannula

At the start of the recording period one nas
passage usually had a high resistance to a
flow when compared with the other and th
resistance of each nasal passage gradually i
creased during the first hour of recording
Spontaneous changes in nasal resistance wer
recorded in all of the pigs and a typical exam
ple of the changes observed in one pig is show
in Fig 2 These were usually slight increase
in nasal resistance of 1–5 min duration (se
right nasal passage, Fig 2) accompanied b
changes in the depth and rate of respirati
and slight movements of the limbs which cou

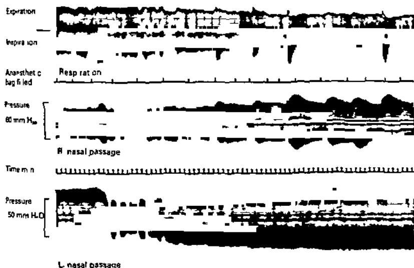


Fig 2 Pen recorder trace of pressure changes in nasal cannulae and changes in respiration Spontaneous changes in nasal resistance are apparent in both nasal passages (see text) (Pig no 36)

not be related to any change in the depth of anaesthesia or to changes in the concentration of carbon dioxide in the inspired and expired gas in the anaesthetic circuit The spontaneous changes in nasal resistance were often repeated at regular intervals with regular changes in respiration

In 10 of the 13 pigs reciprocal changes in nasal resistance occurred with the resistance of one nasal passage increasing as the resistance of the other passage decreased (see Fig 3) The reciprocal changes in resistance started at the same time in each nasal passage and occurred over a period of approximately 30 min These changes in resistance occurred only once or twice during the 4–5 hour recording period and did not usually occur in the first hour of recording This reciprocal activity of the nasal mucosa was observed in 25 out of 31 experiments on 10 pigs

In 4 of the pigs nasal resistance was monitored on three occasions before section of the right cervical sympathetic nerve and on two occasions after nerve section In 3 of the pigs, clamping of the nerve or electrical stimulation of the cut peripheral end caused a marked de-

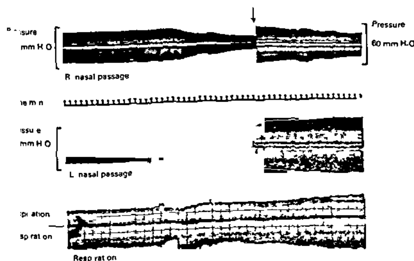


Fig 3 Pen recorder trace of pressure changes in nasal cannulae and changes in respiration Reciprocal changes in nasal resistance are apparent (see text) Note change of gain for the upper trace point marked with ↓

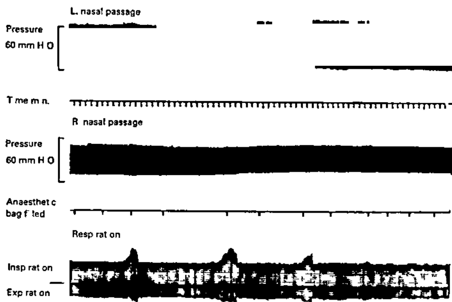


Fig 4 Pen recorder tra pressure changes in nasa cannulae and changes in piration taken from an e periment 14 days after section of the right cerv sympathetic nerve (Pig r 36) Compare with traces from preoperative experi ments in Figs 2 and 3

crease in the resistance of the ipsilateral nasal passage, and in two of the pigs a slight decrease in the resistance of the contralateral nasal passage was also observed In the fourth pig electrical stimulation of the cervical sym pathetic nerve caused only a slight decrease in the resistance of the ipsilateral nasal passage and had no effect on the contralateral passage and it is probable that the majority of the sym pathetic fibres to the nose escaped section in this pig In the preoperative experiments, spontaneous changes in nasal resistance we observed and reciprocal changes in nasal r sistance were recorded in 9 out of 12 expe ments on the 4 pigs All four pigs develop post operative ipsilateral ptosis and hyper mia of the ear After nerve section no recipr cal changes in nasal resistance were observ and spontaneous changes in nasal resistan were rare and usually limited to the nasal pa sage contralateral to the nerve section Fig is a typical record of the nasal resistance aft

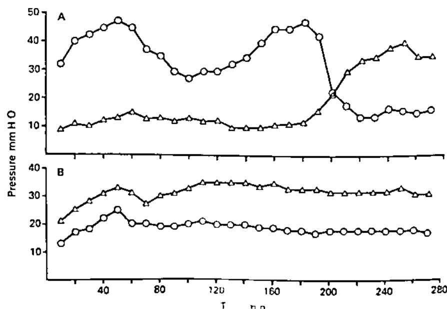


Fig 5 Graphs to illustrat changes in nasal resistan (A) before section of the right cervical sympathe nerve (B) 28 days after nerve section (Pig no 36
O Right nasal passage
Δ left nasal passage

nerve section and it should be compared with Figs 2 and 3 which are records from the same pig before nerve section The gradual increase in the resistance of both nasal passages during the first hour of recording still occurred after nerve section yet after this change the resistance of each passage was relatively constant Fig 5 compares the changes in resistance observed before and after nerve section and demonstrates the low resistance of the right nasal passage after the operation which is typical of the results as no chronic increase in nasal resistance was observed on the right side

DISCUSSION

The results demonstrate that changes in nasal resistance may be recorded in the anaesthetized pig by pumping air through the nasal passages and recording pressure changes in nasal cannulae The pump moves air to and fro in the nasal passages and this overcomes the problem of drying of the nasal mucosa which would occur with a continuous flow of air The posterior nares cannula formed a very low and constant resistance in series with the two nasal passages and this ensured that changes in the pressure recordings from the nasal cannulae were directly related to the state of engorgement of the erectile tissue of the nasal mucosa

Three types of activity were noted in the recordings of nasal resistance Type (1) was a gradual increase in the resistance of each nasal passage over the first hour of recording type (2) was spontaneous increases or decreases in nasal resistance often with a regular periodicity of 5–10 min and usually associated with changes in respiration type (3) was a reciprocal activity with the resistance of one nasal passage increasing as the resistance of the other side decreased

The gradual increase in nasal resistance (type 1) was observed in all of the pigs and this may be due to condensation of water within the nasal passages as during the experiment the air within the pump became saturated with water and it was necessary to drain the cannulae of condensation every 2 hours The increase in nasal resistance might also be explained by some action of the anaesthetic agent

The spontaneous changes in nasal resistance (type 2) were accompanied by changes in respiration Changes in nasal resistance in man have been shown to occur in an integrated way with changes in respiration in response to exercise and changes in arterial pCO_2 (Dallimore & Eccles 1977) and it appears that the respiratory centre may be closely linked to other areas of the brain stem which regulate the activity of the autonomic innervation of the respiratory tract Thus one would expect that changes in respiration would be accompanied by changes in nasal resistance The changes in respiration in the pig may be due to depression of the respiratory centre by the anaesthetic agent and a periodic escape from this depression due to gradual build up of carbon dioxide However any build up of carbon dioxide would be expected to cause a simultaneous decrease in resistance of each nasal passage (Tatum 1923 Dallimore & Eccles 1977) and this was not observed in the present experiments Therefore the results are not fully explained by this hypothesis

The reciprocal changes in nasal resistance (type 3) have been previously reported to occur in the pig (Eccles & Maynard 1975) and are similar to those observed in the human nasal cycle (Stoksted 1953) The changes in resistance are believed to be regulated by changes in the activity of the autonomic innervation of the erectile tissue and this activity is regulated from a nasal centre in the medulla or hypothalamus (Stoksted 1953 Eccles 1977) There is some evidence that the sympathetic nervous system has a major role in regulating the nasal cycle (Stoksted & Thomsen 1953) and this is based on local anaesthesia of the stellate ganglion in man In the present study complete section of the cervical sympathetic nerve was possible and recordings made on the pigs before and after dec

tion of the superior cervical ganglion Section of the right cervical sympathetic nerve abolished the reciprocal changes in nasal resistance and almost completely eliminated the spontaneous changes in nasal resistance, in both nasal passages The bilateral effect of nerve section may be explained by crossover of sympathetic fibres (Wilson & Yates, 1975) although only a fraction of the sympathetic fibres appear to cross over in the pig Another possibility is that sensory fibres have a role in regulating nasal resistance and that ipsilateral nerve section causes a bilateral disturbance Immediately after nerve section there was a gradual increase in the resistance of the ipsilateral nasal passage indicating a constant sympathetic tone A similar result has been reported in man (Beickert, 1950, Stoksted & Thomsen, 1953) However, nerve section did not cause a chronic increase in nasal resistance in the pigs and the development of decentralization supersensitivity may explain the low resistance observed in the right nasal passage in post operative experiments (Malm, 1974)

ACKNOWLEDGEMENTS

Thanks are due to Mr H L Nash and Mr H J Teagle for technical assistance and to the Mechanical Workshop of the Department of Physiology for the construction of the nasal pump and cannulae

ZUSAMMENFASSUNG

In den beiden Nasengangen narkotisierter Schweine wurde der Widerstand gegen einen Luftstrom gemessen in dem Luft durch die Nase gepumpt und Druckanderungen in Nasenkanulen gemessen wurden Bei der Mehrzahl der Schweine wurde ein Nasenzyklus beobachtet Es wurden drei Typen von Nasenwiderstands Veranderungen beobachtet deren mogliche Ursachen besprochen werden Ein Durchschneiden des rechten N sympathicus cervicalis fuhrte zu einer Aufhebung der zyklischen Veranderungen des Nasenwiderstandes in den beiden Nasengangen Die beschriebene Methode könnte sich für die Untersuchung der Physiologie des Nasenzyklus als wertvoll erweisen

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THE NASAL MUCOSA DURING LONG-TERM TREATMENT WITH BECLOMETHASONE DIPROPIONATE AEROSOL

A Light and Scanning Electron Microscopic Study of Nasal Polyps

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Abstract Blind examination by light and scanning electron miscroscope of a total of 142 biopsies from nasal polyps was carried out before and after continuous intra nasal treatment with beclomethasone dipropionate aerosol 400 μg/day for a period of 9–36 months The degree of tissue oedema the number of infiltrating eosinophil cells and the number of goblet cells in the epithelium decreased significantly There was no change in the type of surface epithelium during treatment and no tendency towards squamous cell metaplasia The study suggests that beclomethasone dipropionate treatment for a period of a few years will not cause atrophic rhinitis

Since the introduction of beclomethasone dipropionate (Bdp) aerosol on the British and the European market 2–4 years ago it has become a well established and widely used drug for the management of hay fever, perennial rhinitis and nasal polyposis A series of reports, demonstrating the efficacy of this treatment have appeared (reviewed by Brogden et al , 1975) As the active component is a highly potent corticosteroid with topical activity, it is obvious that the potential risk of local side effects needs consideration, and a study of the Bdp treated mucous membranes very relevant

Dermatological experiences with skin atrophy following corticosteroid treatment suggest that the development of atrophic rhinitis following intranasal Bdp therapy is a possibility and this worries otorhinolaryngologists A characteristic feature of atrophic rhinitis is squamous cell metaplasia of the surface epithelium, demonstrable by both the light (Holopainen, 1967) and the scanning electron microscope (Mygind et al , 1974*a*)

This article presents the results of light and scanning electron microscopy of the nasal mucosa before and after 9–36 months' treatment with Bdp

MATERIAL & METHODS

Following a blind short term investigation of the efficacy of intranasal Bdp in adults with nasal polyposis (Mygind et al , 1975) 33 out of 35 patients continued for a 9–12 months controlled period (Pedersen et al , 1976) with 400 μg Bdp/day as the only treatment Nasal biopsies were taken before and on the last day of the treatment period 21 were treated for 9 months and 12 for 12 months Final biopsies were taken after 36 months in 9 patients who had used Bdp continuously in a daily dosage of 200–400 μg

Two 4–8 mm large mucosal biopsies were taken by forceps or snare from an anterior polyp Local analgesia was not used All biopsies were immediately fixed in a 4°C cold fixative which in the case of light microscopy, was a buffered 4% formalin solution The specimens were embedded in paraffin and 4–6 μm thick sections cut Haematoxyl ...

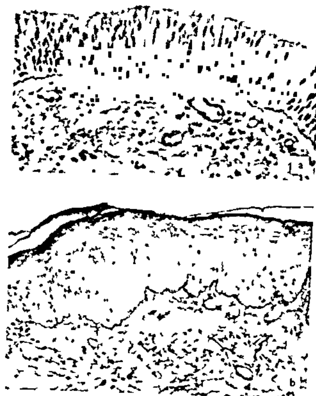


Fig 1 Light microscopic illustration of a pseudostratified epithelium (*a*) and a squamous epithelium (*b*) which correspond to score 100 and score 0 respectively in the lind examination of nasal biopsies before and after be ethasone dipropionate treatment for one year

amine dyed sections were prepared from each specimen

After fixation for 1–7 days in 5% glutaralde hyde in 0 03 M sodium cacodylate (osmolarity measured to 580 mOsm/kg) the biopsies for scanning electron microscopy were trans erred to 0 15 M sodium cacodylate (osmolari y measured to 295 mOsm/kg) They were then passed through a series of acetone solutions rom 10–100% and transferred to liquid CO_2 n an E 3000 Polaron critical point drying ap paratus After drying the specimens were mounted on stubs and a conductive layer of old deposited on them in a vacuum evap rator Finally the dehydrated gold coated pecimen was studied in a Cambridge Stereo can S2 or a Jeol JSM 35 microscope All biop es except those taken after 36 months of treatment, were coded and examined blind in both the light and the scanning electron microscope

A light microscope description of the appearance of the blood vessels and the glands was made based on haematoxylin-eosin and PAS stained sections and a description of the epithelial basement membrane was based mainly on the silver staining of the reticulin fibrils In elastin and in van Gieson sections only traces of elastic and collagen fibrils were found in the polyp tissue, so our study cannot give any information about possible Bdp-induced changes in these structures The degree of tissue oedema was evaluated on a semi quantitative scale (+=1, ++=2, +++=3) All eosinophils within one square millimeter were counted and so were the goblet cells in 1 mm of the surface epithelium Based on the type of the surface epithelium each biopsy was given a score value from 0 (all epithelium of the squamous type) to 100 (all epithelium of the pseudostratified type) (Figs 1, 2)

In the scanning electron microscope a short description of the mucosal surface was given and two low power (×800) and two high power (×3200) micrographs of representative areas were taken The description and micrographs of each biopsy were used to give a score value before the code was broken The score system used is illustrated in Figs 3 and 4 Interpolation between the scores illustrated was necessary, when a biopsy was covered by

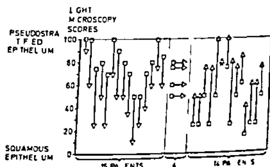


Fig 2 Results of blind light microscopic examination of nasal biopsies before (□) and after beclomethasone dipropionate treatment (Δ)

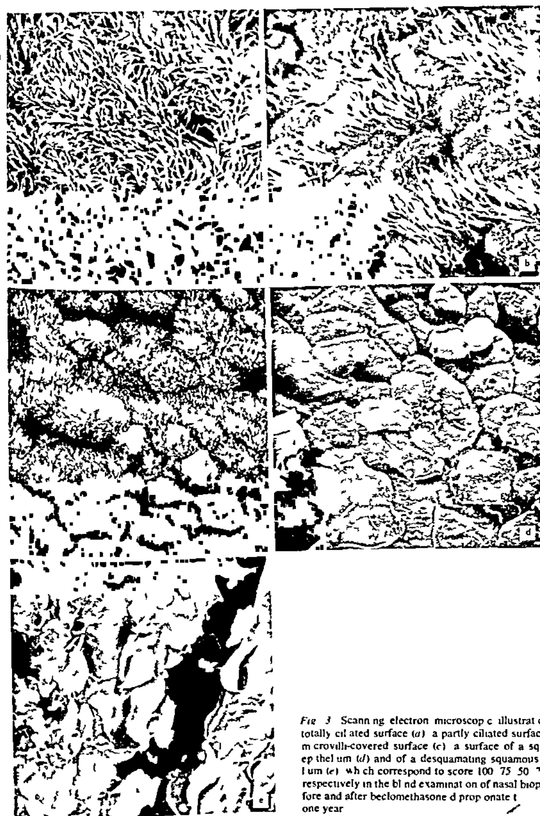


Fig 3 Scanning electron microscopic illustration of a totally ciliated surface (*a*) a partly ciliated surface (*b*) a microvilli-covered surface (*c*) a surface of a squamous epithelium (*d*) and of a desquamating squamous epithelium (*e*) which correspond to score 100 75 50 25 an respectively in the blind examination of nasal biop fore and after beclomethasone dipropionate t one year

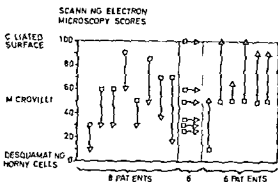


Fig 4 Results of blind scanning electron microscopic examination of nasal biopsies before (□) and after (Δ) beclomethasone dipropionate treatment

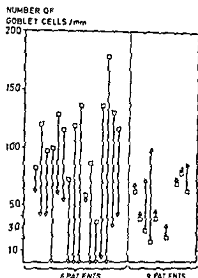


Fig 6 Number of goblet cells in nasal epithelium before (□) and after (Δ) beclomethasone dipropionate treatment

epithelia of varying character Due to this the grading seems very detailed and exact but in fact it was merely semiquantitative

For statistical calculations a paired Wilcoxon sign rank test was used Only for the number of eosinophils and goblet cells the figures were transformed into logarithms In no case did the result of a Wilcoxon test differ significantly from that of a paired *t*-test

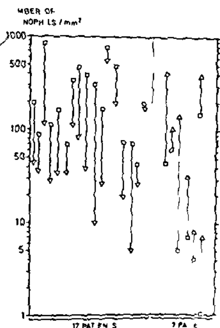


Fig 5 Number of eosinophils in polyp tissue before (□) and after (Δ) beclomethasone dipropionate treatment

RESULTS

Thirty-three pairs of biopsies were fit for light microscopy and 20 pairs for scanning electron microscopy Some biopsies were discarded because, one or both of a pair did not contain enough epithelium, the biopsy was covered by mucus, or for other technical reasons

Light microscopy showed the epithelial basement membrane to be of varying thickness without any consistent changes during the treatment period The appearance of the sparse blood vessels did not change significantly during treatment and no perivascular haemorrhages were observed Before and after treatment most glands were pathological containing only a few cystically dilated and degenerated branches The huge amounts of tissue oedema decreased significantly during Bdp treatment ($p<0.05$) (Table I) and so did the number of infiltrating eosinophils in lamina propria ($p<0.02$) (Fig 5), and also the number of goblet cells in the epithelium ($p<0.01$) (Fig 6)

Figs 2 and 4 show the changes in the type of surface epithelium from the pretreatment to the post-treatment biopsy within the single pairs While the epithelium in 23 pairs of biopsies changed in the direction of a squamous epithelium, it changed in the direction of a



Fig 7 Scanning electron micrograph of a dry surface with many epithelial cells expelled For further interpretation refer to text

pseudostratified epithelium in 20 pair of biopsies and no change occurred in 10 pairs This blind, quantitative study did not disclose any consistent tendency towards the development of squamous cell metaplasia A single post-treatment biopsy deserves further attention for as seen in Fig 7 the surface of this biopsy appeared unclean" and dry with many cells expelled Bdp may possibly account for this as this patient had dryness of the mucosa in the anterior part of the nasal cavity following treatment

The biopsies taken after 36 months treatment did not differ significantly from the pre treatment biopsies with regard to the type of epithelium but this examination was not blind

DISCUSSION

The significant reduction in tissue oedema of nasal polyps following Bdp treatment is consistent with the clinical observation that long term treatment has some shrinking effect on polyps (Pedersen et al 1976) As systemic corticosteroid therapy considerably diminishes the number of nasal eosinophils in hay fever (Mygind & Johnsen 1977) ... notable that an equal effective, but topically administered, corticosteroid has a less pronounced effect on polyp eosinophils This is in accordance with the observation that topical treatment produces only a slight, if any, reduction of eosinophilia in nasal smears (Sørensen et al , 1976, Malm & Wihl, 1976) It seems probable that Bdp is active only in the superficial layers of the mucosa with little effect on the cell diapedesis through the blood vessels

The decreased number of goblet cells following Bdp treatment, may be a direct effect of the topically applied corticosteroid, but it seems more likely that the increased nasal patency during treatment is the direct cause Proliferation of goblet cells is evoked by nasal blockage (Hilding & Hilding, 1970), and in the anterior part of the nose the number of goblet cells is found to be significantly higher in perennial rhinitis patients, especially in patients with pronounced nasal blockage than in normal subjects (Mygind et al , 1974)

Neither light microscopy nor scanning electron microscopy of a total of 142 nasal biopsies showed a change of the surface epithelium from a pseudostratified to a squamous type Thus microscopic studies suggest that continuous Bdp treatment, for up to 2½ years does not induce atrophic rhinitis This is in accordance with the rhinoscopic findings in patients (Pedersen et al , 1976)

It might seem hazardous to draw such conclusions on small mucosal biopsies for the character of the epithelium varies considerably from area to area within the nasal cavity (Mygind 1977) However single mucosal

Table I *Tissue oedema before and after beclomethasone dipropionate treatment for one year*

	Mean of pretreatment values	Mean of posttreatment values
Degree of tissue oedema (+ = 1 ++ = 2 +++ = 3)	2 4	1 6



Fig 8 Using a cast of a human nose it is illustrated that the distribution of a drug delivered from a metered dose freon propelled pressurized aerosol is uneven as it hits the mucosa like a bullet from a gun

biopsies from patients with atrophic rhinitis have shown epithelial pathology (Holopainen, 1967, Mygind et al, 1974*a*) Therefore, we feel that the large number of biopsies studied would have disclosed any significant drug induced changes Based on morphologic studies of polyp surfaces (Mygind, 1977) it is likely hat the results obtained with polyp epithelium ectly can be applied to the ordinary nasal ithelium

The cause of spontaneously developing atrophic rhinitis is unknown, although it has been demonstrated that a lack of antiproteolytic enzymes might be of significance for the tissue damage (Reichert & Hochstrasser, 1972) It is common knowledge that extensive space-creating operations such as turbinectomy and ethmoidectomy may cause squamous cell metaplasia and intranasal crusting It is also known that nasal polyps due to pressure, can break the nasomaxillary suture and increase the distance between the septal and the lateral wall of the nose Such a transformation of the normal slit like cavity to a broad tube will invariably increase the risk of squamous cell metaplasia and crusting Consequently any effective treatment of severe nasal polyposis may indirectly involve a risk of atrophic rhinitis Chronic nasal blockage favours pseudostratified epithelium (Mygind et al, 1974*b*) and prevents the development of atrophic rhinitis, but is intolerable to the patient When improvement of nasal patency is needed it seems reasonable to suggest that conservative surgical treatment (polypectomy) followed by controlled Bdp therapy involves less risk of atrophic rhinitis than a radical surgical procedure such as ethmoidectomy

Corticosteroids affect mainly connective tissue and the direct effect on epithelium is weak or absent (Azarnoff, 1975) One would expect should it occur, a Bdp induced atrophic rhinitis to be due to the steroid s effect on the lamina propria producing squamous cell metaplasia in the presence of an abnormally patent nasal airway The initial step in such a process could not be studied by us as the polyps only contained traces of connective tissue fibrils in the lamina propria However a recent transmission electron microscopic study of the connective tissue fibrils beneath the pharyngeal and the bronchial surface epithelium of asthma patients treated with Bdp for 12–18 months did not show any quantitative or qualitative abnormalities following treatment (Andersson et al, 1977)

Although neither rhinoscopic nor microscopic studies have suggested the development of atrophic rhinitis, some Bdp-treated patients may develop dryness of certain areas of the nasal mucosa and also a single crust (Pedersen et al, 1976) In our experience these changes have not been serious and have been reversed, once the treatment has been stopped These changes which may be followed by haemorrhagic spotting occur on the anterior part of the septum, on the front edge of the inferior and the middle turbinates and on the anterior surface of an anterior polyp As illustrated in Fig 8 these are the areas receiving the impact of the aerosol The uneven distribution of a drug, delivered from a pressurized aerosol may also imply that other areas of the mucosa get subtherapeutic doses of it Therefore, it may be worthwhile considering oth

modes of administration and delivery systems for Bdp (Mygind, 1977)

Until now microscopic studies of the nasal pharyngeal and bronchial mucosa have not disclosed any serious local side effect following topical Bdp treatment (Andersson et al , 1977, Brain & Poynter, 1977, Jorde & Werdermann, 1977, Thiringer et al , 1975) Investigations of the nasal and the pharyngeal mucosa may be of special importance, for the steroid dosage per square centimeter is at least 100 times greater in these areas than in the bronchi Therefore, any local side effects are likely to appear first in the nasal or pharynx mucosa As inspection of the nasal mucosa is easy, it should be carried out regularly in all patients on continuous Bdp treatment both as a safety factor and to increase our knowledge of topical corticosteroids' action on the airway mucosa

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The scanning microscopes were kindly placed at our disposal by the Institute for Historical Geology and Paleontology Copenhagen (H J Hansen M Sc) and the Department of Pathology Hvidovre Hosp tal (P Christoffersen M D) The specimens for light microscopy were prepared with skill by Mrs J Nørgaard Glaxo Copenhagen kindly supplied all aerosols used

ZUSAMMENFASSUNG

142 nasale Polypen wurden blind vor und nach einer 9–36 monatigen intranasalen Behandlung mit Beclomethasone dipropionate aerosol 400 μg/tgl licht und elektron mikroskopisch untersucht Der Grad des Gewebsodems sowie Anzahl an eosinophilen Zellen und epithelialen Becherzellen waren deutlich herabgesetzt Wahrend des Behandlungsverlaufes konnte man keinerlei Veranderungen des Oberflachenepithels und keinerlei Tendenz zur Plattenepithelmetaplasie konstatieren Aus der Untersuchung kann man schließen daß eine durch wenige Jahre verabreichte Behandlung mit Beclomethasone dipropionate nicht zu atrophischer Rhinitis führt

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IDENTIFICATION OF β-ADRENOCEPTORS AND HISTAMINE RECEPTORS IN THE CAT NASAL VASCULATURE

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Abstract The responses of the nasal capacitance vessels of the cat were recorded following intra arterial injections of β adrenoceptor agonists and histamine H_1 and H_2 receptor agonists Isoprenaline evoked vasodilation and propranolol (β adrenoceptor antagonist) caused a paral [illegible] onse [illegible] nist) [illegible] all evoked vasodilation Metiamide (H_2 antagonist) produced a further small parallel shift to the right of the histamine log dose–response curve after the administration of mepyramine (H_1 antagonist) It is concluded that the nasal capacitance vessels of the cat contain β_2-adrenoceptors and H_1 and H_2 histamine receptors

[illegible]though several workers have attempted to [illegible]entify β adrenoceptors in the nasal blood [illegible]essels, their existence remains a subject of some debate Hall & Jackson (1968) could not demonstrate β adrenoceptors in the nasal vasculature of the dog, nor could Anggård & Edwall (1974) in their studies on the exchange vessels of the cat However, in another series of experiments, evidence was obtained in the cat to suggest that β-receptors of the β_2 type are present (Malm, 1974*b* 1977) One of the aims of the present investigation was to re-examine this problem

In contrast to the several studies on adrenoceptors, the histamine receptors that are also present within the nasal vasculature have received little attention despite the widespread use of histamine H_1-antagonists in allergic rhinitis However, Bentley & Jackson (1970) showed that exogenous histamine and endogenous histamine released by compound 48/80 produced nasal congestion in the dog Th[illegible] was not antagonized by H_1-receptor antag[illegible]nists at doses used clinically Recently, th[illegible] study of histamine receptors has been greatl[illegible] facilitated by the development of specific ag[illegible]nists and antagonists for H_1- and H_2-recepto[illegible] (Black et al , 1972, Flynn & Owen, 1975) an[illegible] both types of receptors have been shown t[illegible] participate in the responses to histamine in [illegible] number of vascular beds (Flynn & Owe[illegible] 1975, Powell & Brody, 1976) In order to ident[illegible]fy the types of histamine receptors present [illegible] the nasal vasculature we have studied the e[illegible]fects of some compounds active on both hist[illegible]mine H_1- and H_2-receptors

METHOD

Cats of either sex weighing 2–3 kg we[illegible] anaesthetized with sodium pentobarbiton[illegible] (40 mg kg $^{-1}$), the trachea and the cephalic ve[illegible] of one fore-limb cannulated and the systemi[illegible] blood pressure recorded from a femoral arter[illegible] by a Bell & Howell pressure transducer (typ[illegible] 4-327-L221) Constrictions and dilatations [illegible] the nasal blood vessels were recorded respec[illegible]tively as decreases and increases in pressur[illegible] in the sealed right nasal cavity using a cannul[illegible] inserted into the nostril and a type 4 327 L22[illegible]

This work was supported by a United Kingdom Med[illegible] Research Council Project Grant to Dr H Wilson

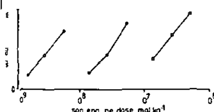


Fig 1 Effects of propranolol on the log dose–response curve for isoprenaline acting on the capacitance vessels of the cat nasal mucosa ○ Responses in the absence of an agonist ● responses in the presence of $3\ 4\times10^{-7}$ mol kg propranolol □ responses in the presence of $3\ 7\times10^{-6}$ mol kg propranolol Vasodilation is expressed as increases in pressure in the sealed nasal cavity (in cm H O) and each point is the mean of three responses

Bell & Howell pressure transducer (Wilson & Yates 1975) This method only records the overall vascular changes in the nose and will mainly reflect the changes in the capacitance vessels (Anggård & Edwall 1974 Malm 1974*a*) Recordings were made on a Devices M2 recorder

Agonists were injected in 50–400 μl isotonic saline into the right external carotid artery by means of a cannula placed in the right lingual artery Injection of up to 600 μl saline by this route had no effect either on the nasal vessels or on the systemic blood pressure Antagonists were administered into the cephalic vein as a slow intravenous injection with the exception of metiamide which was given by a continuous infusion at a flow rate of 0 12 or 0 29 ml min $^{-1}$

The effects of antagonists are expressed in terms of the mean dose ratio ± S E M i e the factor by which the agonist dose must be increased in the presence of an antagonist in order to produce vasodilator responses of the same magnitude as those obtained before addition of the antagonist Tests of significance were made by Student s paired *t* test

The following drugs were used isoprenaline hydrochloride (Pharmax) salbutamol sulphate (Allen & Hanbury) propranolol hydrochloride (ICI) phentolamine mesylate (CIBA) mepyramine maleate (May & Baker) metiamide 2 (2 aminoethyl)pyridine and 4 methylhistamine (SK & F Laboratories)

RESULT

β-adrenoceptor studies

Injections of isoprenaline in 4 cats produced dose related vasodilation This response of the nasal vessels to isoprenaline was antagonized by the administration of propranolol (Fig 1) The mean dose ratio for isoprenaline produced by $3\ 4\times10^{-7}$ mol kg $^{-1}$ propranolol was 17 3± 5 6 At $3\ 7\times10^{-6}$ mol kg $^{-1}$ propranolol the isoprenaline dose ratio was 160+8 9

Nasal vascular responses to injections of salbutamol ($7\ 0\times10^{-11}$ to $7\ 0\times10^{-9}$ mol kg $^{-1}$) were compared with those produced by isoprenaline ($1\ 6\times10^{-11}$ to $8\ 0\times10^{-10}$ mol kg $^{-1}$) in 10 cats Both drugs evoked vasodilation and the log dose response curves were approximately parallel in all the experiments The equipotent molar ratio of salbutamol/isoprenaline was 8 8±1 1

Histamine receptor studies

Injections of histamine 2 (2 aminoethyl)pyridine (an H_1 agonist Flynn & Owen 1975) and 4 methylhistamine (predominantly an H_2-agonist Flynn & Owen 1975) produced dose dependent vasodilation in the nasal vessels The log dose–response curves were parallel (Fig 2) and equipotent molar ratios were determined in 11 cats The ratio of the potency of 4 methylhistamine/histamine was 17 2±3 1

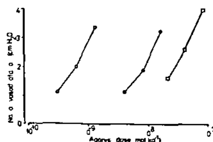


Fig 2 Log dose–response curves for three agonists active at histamine receptors ○ Histamine ● 4 histamine □ 2-(2 aminoethyl)pyridine Each point is mean of three determinations

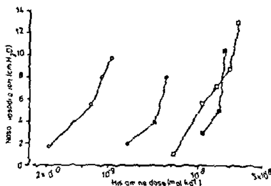


Fig 3 Log dose histamine-vasodilation curves showing the effects of increasing doses of mepyramine O Control responses ● □ and ■ responses in the presence of 2 4×10⁻⁷ 2 6×10⁻⁶ and 7 2×10⁻⁶ mol kg⁻¹ mepyramine respectively Each point is the mean of three responses

whilst that for 2 (2-aminoethyl)pyridine/histamine was 45 0±2 9

Mepyramine antagonized the effects of histamine (Fig 3) but the use of this antagonist was complicated by the intense vasoconstriction it produced in the nasal vasculature In the experiment illustrated in Fig 3 injection of 2 6×10⁻⁶ mol kg⁻¹ mepyramine produced a decrease in pressure in the sealed nasal cavity which reached a maximum of 7 4 cm H_2O within 3 min and then declined slowly, dis- ... after 20 min In 2 animals it proved ... le to record responses of the nasal vessels to agonists after this dose of mepyramine because of the intense vasoconstriction it produced Accordingly, the dose was restricted to 1 2×10⁻⁶ mol kg⁻¹ in later experiments This dose produced a mean dose ra of 7 9±1 3 in 6 cats and subsequent infusion metiamide (2 3×10⁻⁶ mol kg⁻¹ min⁻¹) gav further small (though significant $p<0$ rightward shift in the histamine log dose-response curve in all the animals (mean d ratio 9 6±3 5) One experiment is shown Fig 4

Mepyramine (1 2×10⁻⁶ mol kg⁻¹) in 5 c produced only a very small mean shift in log dose–response curve to the H_2-agonist methylhistamine (mean dose ratio 1 5±0 but subsequent infusion of metiam (2 3×10⁻⁶ mol kg⁻¹ min⁻¹) produced a parallel shift (mean dose ratio 57 2±12 7) contrast, the response to 2-(2 pyridine after mepyramine was biphasic w a brief non dose related vasodilation follow by vasoconstriction of greater duration T vasoconstriction could be considerably duced or abolished by intravenous phent amine (2 6×10⁻⁶ mol kg⁻¹)

Infusion of metiamide alone, at rates 9 3×10⁻⁷ and 2 3×10⁻⁶ mol kg⁻¹ min⁻¹ or had significant effects ($p<0$ 05) on the l dose–response curve for 4 methylhistamin With this agonist the shift in the curve w parallel for each infusion rate and the fast rate produced the larger shift The mean do ratios were 16 4±26 9 and 68 4±96 8 respe tively In the 2 cats that received mepyrami during the faster metiamide infusion there w a further rightward shift of the curve (me

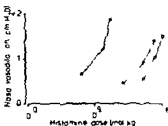


Fig 4 Effects of antagonists on log dose response curves for histamine O Responses of the nasal capacitance vessels to histamine before administration of antagonists ● responses after 1 2×10⁻⁶ mol kg mepyramine □ responses in the presence of mepyramine (1 2×10⁻⁶ mol kg⁻¹) and metiamide (2 3×10⁻⁶ m l kg m n)

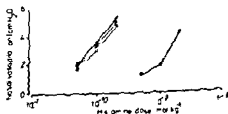


Fig 5 Effects of antagonists on the log dose-respon curves for histamine O Responses before administra of antagonists ● responses after metiamide (9 3×10 mol kg⁻¹ min⁻¹ □ responses after metiamide (2 3 10⁻⁶ mol kg⁻¹ min⁻¹) ■ responses after metiamide (2 3 10⁻⁶ mol kg⁻¹ min⁻¹) and mepyramine (1 2×10⁻⁶ m kg⁻¹)

ose ratio 151) There was a significant shift in the log dose–response curve to histamine only when mepyramine was administered during metiamide infusion (Fig 5) The log dose–response curve to 2 (2 aminoethyl)pyridine was not significantly altered by either infusion of metiamide but again became biphasic when mepyramine was given during the infusion

DISCUSSION

Previous pharmacological investigations of the nasal vasculature have demonstrated the presence of α adrenoceptors in this vascular bed (Hall & Jackson, 1968, Malm, 1974*a*) but there is disagreement concerning the presence of β adrenoceptors β receptors were not detected in the capacitance vessels of the dog (Hall & Jackson, 1968) or in the exchange vessels of the cat (Anggård & Edwall, 1974) On the other hand, Malm (1974*b*) found evidence for the presence of β receptors in the resistance vessels of the cat nasal mucosa but his results for the capacitance vessels were inconclusive

The experiments with isoprenaline reported here clearly show that dilation of the nasal capacitance vessels can be produced by close arterial injection of this β agonist Furthermore, this response is antagonized in a dose-dependent manner by propranolol, a β antagonist Dilation of the vessels was also produced by administration of salbutamol and comparison of the potencies of the two agonists gave a mean isoprenaline/salbutamol activity ratio of 8 8 which is within the range of activity ratios for tissues presumed to contain mainly β_2 receptors (Schild 1973) These results show that the nasal capacitance vessels of the cat contain β adrenoceptors of the β_2 subtype which is the subtype that Malm (1974*b*) concluded were present in the resistance vessels

Further pharmacological investigation of the nasal capacitance vessels shows that both types of histamine receptor are present since H_1 and H_2 agonists produce vasodilation which can be antagonized by the appropriate antagonists 2-(2 aminoethyl)pyridine acts specifically at H_1-receptors and the potency ratio relative to histamine in the nasal vascular bed corresponds closely to the value of 43 7 reported for other vasculatures (Flynn & Owen, 1975) Also, whilst 4 methylhistamine is not specific to H_2-receptors the equipotent molar ratio of 17 2 in the nasal mucosa is intermediate between that for H_1- and H_2 receptors elsewhere (200 and 2 67 respectively, Flynn & Owen, 1975)

The vasoconstrictor response to 2 (2 amino ethyl)pyridine following mepyramine treatment may be due to the release of catecholamines from the adrenal medulla as a result of the large doses employed passing through the nasal vessels into the systemic circulation It is also possible that it represents a direct sympathomimetic action

The antagonists also produce results closely comparable to those from other vascular beds (Flynn & Owen, 1975) Thus the H_2 receptor antagonist metiamide had no effect on the response to histamine unless it was given in the presence of the H_1-antagonist mepyramine Together the antagonists metiamide and mepyramine produced greater antagonism than either one acting alone when used against histamine or 4-methylhistamine, agonists active at H_1- and H_2 receptors Also mepyramine appeared to have a maximum effect against histamine with a dose ratio of between 16 and 19 at a dosage of $2\ 6 \times 10^{-6}$ mol kg^{-1} but the vasoconstriction produced by mepyramine *restricted the experiments that could be performed* The value for the maximum dose ratio corresponds closely to those reported by Flynn & Owen (1975) for the vascular beds of the hind limb (16 3) and superior mesenteric region (13 8) of the cat consistently obtained with $2\ 5 \times 10^{-5}$ mol kg^{-1} mepyramine

The experiments with histamine antagonists suggest that the combined use of H_1 and H_2-antagonists may be of therapeutic value in treatment of allergic rhinitis However, be that histamine antagonism is not

of the effectiveness of H_1-antagonists as has been suggested by Bentley & Jackson (1970) These workers suggested that the direct vasoconstrictor effect could be the mechanism of action of these drugs and experiments in the present study have shown that strong vasoconstriction is produced at doses of mepyramine which produce only moderate blockade of histamine induced vasodilation

In conclusion, the experiments described in this study show that the nasal capacitance vessels of the cat contain β_2-adrenoceptors and H_1- and H_2 receptors for histamine

ACKNOWLEDGEMENTS

Metiamide 2 (2 aminoethyl)pyridine and 4 methylhistamine were the kind gift of Dr D A A Owen of Smith Kline & French Laboratories

ZUSAMMENFASSUNG

Die Wirkungen von intraarteriell verabreichten β Adrenorezeptoragonisten und von Histamin H_1 und H_2 Rezeptoragonisten wurden auf die Kapazitatsblutgefaße der Nasenschleimhaut der Katze untersucht Isoprenalin loste eine Vasodilation aus und Propranolol (β Adrenorezeptor antagonist) hatte eine parallele nach rechts verschobene *Log-Dosis–Wirkungskurve zur Folge* Histamin 2 (2 minoathyl) pyridin (H_1 Agonist) und 4 methylhistamin uptsachlich H_2 Agonist) bewirkten eine Vasodilation etiamid (H_2 Antagonist) bewirkte eine weitere aber geringfugige parallele Verschiebung der Log Dosis Wirkungskurve des Histamins nach rechts nach Verarbreichung von Mepyramin (H_1 Antagonist) Daraus kann man schließen daß die Kapazitatsblutgefaße der Nasenschleimhaut der Katze β_2 Adrenorezeptoren sowie H_1 und H_2-Histaminrezeptoren besitzen

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RHINOLOGICAL FINDINGS FOLLOWING TRANSANTROSPHENOIDAL SURGERY OF THE PITUITARY GLAND

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stract Fifty patients with pituitary gland tumours on om we operated via the transantrosphenoidal route re rhinologically followed up with a view to revealing / conceivable functional impairments incurred by this thod Our investigations included rhinoscopy, endo- ›py of the nasal and paranasal cavities and of the epi arynx X ray controls and probes of the nn olfactorius 1 trigeminus Apart from the technical advantages of nsantrosphenoidal pituitary gland surgery, the results o confirmed its benefit to functional performance

hree rhinological access routes to the sella rcica are predominant in the surgery of pitui ry adenomas (Fig 1)

1 The transseptal route *ad modum* Hirsch 909, 1952), is favoured by neurosurgeons hen modified according to Guiot (1969, 1976), uiot et al (1959, 1967, 1972), Fahlbusch et al 973, 1976), Kautzky et al (1976), Schürmann 974), Samii & Schurmann (1976), unless the ituitary gland tumour is operated on trans- ranially

2 The transethmoido-sphenoidal approach ccording to Chiari (1912) used by a number of hinosurgeons (Nager, 1940, Escher, 1962, 965 1974, Angell James, 1967, Burian, 1967, 969, 1974 1976)

3 The transantrosphenoidal operation nethod according to Denker (1921), which eads via the sinus maxillaris and the ethmoi- lal cell system into the sphenoidal sinuses and o the sella Hamberger (1976) has used this hinosurgical procedure since 1955, and from his pioneer work in pituitary surgery, he and us co workers (1960 1961, 1964, 1969 1974) gathered their experience gained on 450 pa- tients operated on with this method

The aim of this paper is to report our expe- rience, with rhinological findings, following transantrosphenoidal surgery on the sella tur- cica

MATERIAL AND METHOD

Of our own 65 patients treated by transantro- sphenoidal surgery of the pituitary gland (Kar duck & Bock, 1977), we have followed up rhi- nologically 50 patients for periods of from 5 months to 3½ years, at the latest, at regular in- tervals (Fig 2) Case history and ENT diagno- sis ascertained for these patients prior to op eration failed to reveal any pathological condi- tions There were 15 cases of deviation of the septum, though this did not cause any ventila- tory disturbance

RESULTS

In the following, the non desired side effects attributable to this operative technique are re- ported on

Blood crusts seen by means of the rhino- scope in the early postoperative phase follow- ing removal of tamponade, were met with in all cases but could be quickly cleared up by means of unguents

Problems were caused, however, by those crusts appearing in the nasal cavity on the op-

TRANSSEPTAL ROUTE HIRSCH 1910	GUIOT (1957 19 6) 740 CASES
TRANSETHMOIDOSPHENOIDAL ROUTE (CHIARI 1912)	ANGELL JAMES (1960 1967) 350 CASES BURIAN (1961 1977) 330 CASES
TRANSANTROSPHENOIDAL ROUTE (DENKER 1921)	HAMBERGER (1955 1976) 450 CASES



Fig 1 The three common rhinological routes to the sella turcica.

erated side, which formed from dried secretion and which differed distinctly from the a/m in appearance This formation is an unmistakeable sign of a severe impairment of the respiratory epithelium It occurred in 5 patients, and we believe it to be due to a temporary local ischemia caused during operation by pressure exerted by the operating instrument, with consequent injury to the mucosa The crustation stopped in all instances, though it necessitated treatment with unguents and salt water rinsing of the nose for months, plus frequent rhinological controls

Another undesired effect appeared in 2 cases, viz large adhesions of the mucosa of lateral nasal wall to the septum causing severe impairment of nasal ventilation Fine synechiae without disturbance of ventilation were encountered 7 times in the nasal cavity The two adhesions had to be cut through in order to restore nasal ventilation

When using the transantrosphenoidal route the front wall of the ethmoid is opened wide, necessitating in some instances a resection of the posterior part of the septum and of the posterior ends of the conchae, this did not lead in any case to any functional impairment

We paid special attention to liquorrhea and meningitis which may occur postoperatively we found them in one case each as a transient complication However, since changing over to blocking the sella with a bone fragment taken from the facial sinus wall in addition to the usual closure of the sella with a free muscle graft, we have not seen any such complications We found that this bony plate adapts easily during the healing process a fact proved by X ray and endoscopic controls

The sensibility probe of the fifth cranial nerve revealed in all cases a hypesthesia within the supply area of its second branch though this normalized in every instance during the first postoperative weeks No disturbances in the sense of smell occurred

Septum perforation was not found in any of our patients

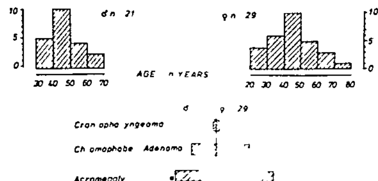


Fig 2 Age sex and diagnosis in 50 cases of transantrosphenoidal surgery of pituitary adenomas

DISCUSSION

Recently Kinnman (1973) has published in a clinical study on 80 patients treated by transantrosphenoidal surgery a report of postoperative local reactions as follows A moderate swelling of the soft tissues corresponding to the antral opening was seen in most patients but it subsided during the course of a week to 10 days A moderate nasal discharge was usually seen during the first 3–4 weeks As a rule the patients also experienced some hypesthesia in the cheek and nasolabial area lasting for periods up to 3 months In one patient an oro-antral fistula persisted postoperatively, but closed spontaneously 4 months after the operation In one patient an indolent swelling below the area of the right lacrimal sac developed one year after the operation and the nasolacrimal duct was obstructed on the same side an isolated ethmoidal mucocele was found In another patient a moderate epiphora due to obstruction of the naso lacrimal duct on the side of the transantrosphenoidal operation has developed 4 years postoperatively Hyposmia or anosmia has never been encountered nor has an atrophic rhinitis developed in any patient Postoperative complications in form of liquorrhea and meningitis were unusual

Fahlbusch (1977), a neurosurgeon who has used the transseptal route found crustation and septum perforation to be the most frequent complications

We are indebted to Burian (1977) for the clearest statement, drawn from his ample case material and controls gathered over many years, according to this a mucocele of the frontal sinus seems to be a very rare occurrence, though a typical complication of the transethmoido-sphenoidal treatment of pituitary gland tumours

For our 50 transantrosphenoidally operated patients endonasal crustation is the predominant complaint Nevertheless we must stress in agreement with the above mentioned authors (Kinnman Fahlbusch Burian) that *meningitis* and *liquorrhea* which are the most dangerous postoperative sequelae, are very rarely met with for all three rhinological operative methods

ZUSAMMENFASSUNG

50 Patienten mit Hypophysentumoren d e wir auf trans antrosphenoidalem Weg operierten wurden rhinologisch nachuntersucht zur Überprüfung der Frage welche Funktionsbeeinträchtigungen als Folge dieser Methode auftreten Die Untersuchungen umfaßten die Rhinoskopie die Endoskopie der Nasenhaupt und nebenhöhlen sowie des Nasenrachens Rö Kontrollen und N olfactorius und N trigeminus-Prüfung Die Ergebnisse zeigten neben den operationstechnischen Vorteilen der transantrosphenoidalen Hypophysenchirurgie auch ihren funktionsschonenden Charakter auf

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ELECTROMYOGRAM OF THE TENSOR TYMPANI MUSCLE IN MAN DURING SWALLOWING

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Abstract Experiments were carried out on 2 patients who underwent an operation for chronic otitis media where upon the tensor tympani muscle was visualized A unipolar platinum electrode was inserted into the muscle belly EMG recordings were made during swallowing and other motor activity Distinct pronounced EMG activity was recorded from both patients every time they swallowed It was concluded that the tensor tympani muscle participates in the act of swallowing and thereby probably contributes to the ventilation of the middle ear

Though the structure of the middle ear muscles, the stapedius and the tensor tympani, was first described more than 400 years ago (Eustachius, 1562, Varolius, 1575) their function in man has not been studied in detail prior to the most recent decades The function of the stapedius muscle has been elucidated in a number of investigations (e g Fletcher, 1962, Borg & Zakrisson, 1974 Zakrisson, 1974) In contrast the function of the tensor tympani muscle in man is much less clear Acoustically induced tensor tympani reflexes have been reported by Terkildsen (1957, 1960), but Klockhoff (1961) considered this phenomenon to be merely a part of a startle response Liden et al (1970) discovered acoustically induced tensor tympani reflexes in 13 % of normal individuals tested Jepsen (1955) and Zakrisson (1975) found a total absence of a tensor tympani reflex upon acoustic stimulation in patients with peripheral facial palsy including stapedius muscle paralysis

The following methods have been used to study the activity of the tensor tympani muscle in man

(*a*) recording of the changes in impedance of the ear (Jepsen, 1955, Klockhoff, 1961)

(*b*) measurement of changes in position of the eardrum (Terkildsen, 1957, Holst et al , 1963, Ingelstedt & Jonson, 1967, Liden et al , 1970)

(*c*) direct visual observation of the tensor tympani tendon (Djupesland, 1964)

(*d*) electromyography (Salomon & Starr, 1963, Djupesland, 1965)

As a rule, tactile stimulation of the regions around the eyes and ears and acoustic stimulation have been used to induce the tensor tympani reflex Tactile stimulation of the external ear region possibly elicits tensor tympani muscle activity in man, which is interpreted by most researchers as one component in a startle response (Klockhoff, 1961, Djupesland, 1964) A tensor tympani muscle contraction having the character of a reflex has been thought to arise even in connection with motor activity in the tensor palati muscle EMG activity in the tensor tympani muscle in association with vocalization and swallowing has been reported by Salomon & Starr (1963) and Djupesland (1965) Tensor tympani muscle activity has been recorded during belching and yawn[illegible] means of volume-flow measurements [illegible] stedt & Jonson, 1967)

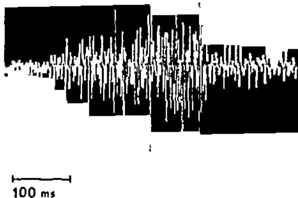


Fig 1 EMG recording obtained during swallowing with a unipolar platinum electrode in the tensor tympani muscle belly and with a reference electrode in the ipsilateral membraneous ear canal

The tensor tympani muscle differs as to anatomical configuration in animals and man In the cat, the muscle consists solely of pars petrosa, while in the rabbit and the guinea pig it includes both a pars petrosa and a pars tubaria, in man only the pars tubaria portion is present (Kirikae, 1960) The tensor palati muscle is embryologically related to the tensor tympani muscle and has the same motor innervation, the trigeminal nerve (Hamilton et al, 1959)

The aim of the present investigation was to study whether the tensor tympani muscle is activated in the act of swallowing

MATERIAL AND METHODS

The experiments were carried out on 2 patients with chronic otitis media with cholesteatoma Radical mastoidectomy was performed in both cases under local anesthesia The amount of anesthetic fluid (1% Xylocaine-exadrine) was kept as low as possible The operation revealed that both patients had cholesteatoma in the entire epitympanum and the middle ear and parts of the ossicles were absent The attic and the middle ear were opened widely with a drill and with the aid of a microscope, cholesteatoma granulation tissue and all remaining parts of the ossicles, except the footplate of the stapes were removed The tendon of the tensor tympani muscle and the processus cochleariformis were identified About 5 mm of the belly of the tensor tympani muscle was visualized by means of drilling away the processus cochleariformis and parts of the septum of the musculotubal canal

EMG recordings from the tensor tympani muscle were obtained with a unipolar platinum electrode inserted into the muscle belly The tip diameter of the electrode was about 10 µm and around parts of it a glass rod had been cast The reference electrode was a stainless steel hypodermic needle inserted into the dorsal part of the ipsilateral membranous ear canal The experiments were carried out in an electrically shielded cage The amplified EMG potentials were recorded on one channel of a 2-channel tape-recorder (Revox A77) and on an ink writer (Grass Polygraph) The activity was also displayed on an oscilloscope (Tektronix 561) The patients were told to swallow repeatedly with minimal movements of the jaw- and face-muscles, to turn their heads, to yawn and move their arms Instructions to the patients were recorded into the taperecorder's second channel The EMG signals were analysed on a later occasion

RESULTS

The results from the 2 patients were similar During rest, no EMG activity was observed During every act of swallowing a burst of motor unit potentials was recorded from the muscle, each burst having a duration of about 300 ms The potentials were distinct spikes clearly standing out from the background activity See the figure, which also shows that a number of motor units within the uptake area of the electrode took part in the activity Yawning and movements of the head gave only weak and diffuse activity, while arm movements induced no activity whatsoever

DISCUSSION

The results from the present study in man showed that the tensor tympani muscle con

racted during swallowing Swallowing is an ct directed by the central nervous system and which the ventilation of the middle ear is in-mately linked by means of the opening of the Eustachian tube The results presented here ndicate that the tensor tympani muscle might lay a role in the ventilation of the middle ear ngelstedt & Jonson (1967) observed that an verage of 7 5 microlitres of gas flowed hrough the Eustachian tube toward the epi-harynx when a tensor tympani muscle con-raction took place Borg (1972) found that ransudate developed in the middle ear of rab-its in which the tensor tympani muscle had een eliminated These observations also sup ort the assumption that the tensor tympani nuscle contributes to the ventilation of the niddle ear cleft

ZUSAMMENFASSUNG

Die Untersuchungen wurden an zwei Patienten unter der Operation von chronischer Mittelohrentzundung durch efuhrt wobei der Musculus tensor tympani freigelegt vurde Eine unipolare Platinelektrode wurde tief in den

at registriert werden Daraus ist zu schließen daß der M tensor tympani am Schluckakt beteiligt ist und *dadurch wahrscheinlich zur Ventilation des Mittelohrs* beiträgt

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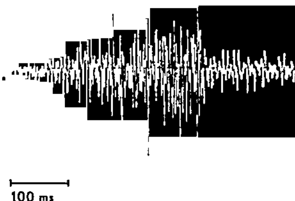


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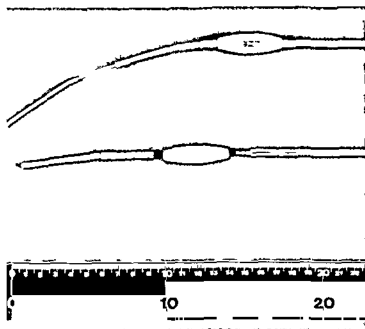


Fig 1 (*Upper*) The guide wire with the soft flexible spring in the distal end and the bougie *itself leading* over the guide (*Lower*) The different parts of the bougie A complete unit consists of 13 dilators from F 21 to F 45

g 2 The Eder–Puestow technique of dilating oesophageal strictures using TV fluoroscopy (SAAB Multiplane)) Oesophagoscopy with introduction of the guide wire (B) The guide wire after the oesophagoscope has been removed (C) The bougie is fitted over the wire (D) the bougie is introduced into the oesophagus



Fig 3 The guide wire in the stomach and the bougie itself in the oesophagus

OWN SERIES

The dilation system used in this trial was described by Puestow (1955) and its use in connection with the flexible oesophagoscope by Lilly & McCaffery (1971) Bennett (1972) Price et al (1974) and Royston et al (1976)

The dilation is performed over a guide wire of steel on the tip of which is fitted a very flexible soft spring (Fig 1) The wire has an external diameter of 2 mm and can be inserted through the biopsy channel in the flexible oesophagoscope The bougie consists of a flexible tip an olive shaped dilator in French 21 to 45 and a flexible staff of 65 cm in length

All the patients in this trial were treated under general anesthesia The procedure is shown in Fig 2 Using a flexible end viewing oesophagoscope (Olympus EF) or gastroscope (Olympus Gif K) the guide wire is passed through the stricture under visual as well as TV fluoroscopy control (Fig 3) When the spring of the wire has reached the stomach

Table I *Oesophageal strictures treated with the Eder–Puestow apparatus*

37 patients	108 dilatations
5 patients with malignant tumour	5 dilatations
32 patients with benign strictures	103 treatments

Table II *Aetiology of oesophageal strictures treated with the Eder–Puestow apparatus*

	No of pats
Malignant tumour	5
Benign stricture	32
Esophageal corrosion	1
Incompetent sphincter (LES)	25
Scleroderma	4
Miscellaneous	2



ig 4 Incorrect technique leading to perforation of the oesophagus with the Eder-Puestow dilator

the scope is withdrawn, and bougies of suitable diameters are led down over the guide wire without risk of perforation

This procedure has now been used in Copenhagen University Medical Centre, Division of Thoracic Surgery, Rigshospitalet over a 2 year period (1974–76) Thirty seven patients underwent treatment (Table I)

The etiology of stricture development (Table II) was incompetent lower oesophageal sphincter in 25 cases, sclerodermia in 4 cases, oesophageal corrosion in one case, miscellaneous reasons in 2 cases and malignant tumour in 5 cases

In these 37 patients a total of 108 dilations were performed with intervals of between 4 days and 6 months (average 2 months) No complications were seen due to this Eder–Puestow procedure

DISCUSSION

Once decision is made to apply bouginage to a patient with stricture of the oesophagus the Eder–Puestow dilation instrument is the instrument of choice The risk attending conventional dilation through a stiff oesophagoscope is perforation with a mortality rate at the same level of mortality for oesophageal resection, when performed at centres using this kind of operation

The dilation treatment with the flexible oesophagoscope can be practicable with a safety hitherto unknown When performed with a pliable and gentle hand, we believe that the procedure is the safest currently known and the risk of perforation hardly greater than with oesophagoscopy with fiberscope itself, i e about 0 05% (Schiller et al , 1972)

Should the staff and bougie push the wire foreward instead of sliding over it the wire will no longer serve as a guide, and perforation can result (Fig 4) It is therefore essential to keep the wire taut and to ensure that the staff is sliding over it

ZUSAMMENFASSUNG

Die große Sicherheit in der Bougiebehandlung enger Ösophagusstrikturen wird erzielt durch die Verwendung eines flexiblen Ösophagoskops wodurch eine Leitsonde a m Puestow eingelegt wird Hieruber wird die Bougie ohne Gefahr von Perforation eingeführt 108 Dilationen wurde ohne Verwicklungen vorgenommen

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Conferences and Meetings

1978 June 1-3 Ordinary meeting of the Bárány Society to be held at the Dept of Otolaryngology, Akademiska sjukhuset, S 750 14 Uppsala, Sweden.

1978 June 1-3 Mayo Clinic Course ' Current Medical and Physiological Aspects of Rhinology Addr Clifford F Lake, Mayo Clinic, Rochester, MA 55901

1978, June 5-16 A two-week Temporal Bone Surgical Dissection Course will be held at the Ear Research Institute in Los Angeles Addr Antonio De La Cruz, 256 South Lake Street, Los Angeles CA 90057

1978 June 19-23 A Postgraduate Course with a Seminar on hypopharyngeal carcinoma and cervical esophagus will be held in Milano Italy Addr Miss Lucia Manfredi Istituto Nazionale Tumori Via G Venezian 1, 20133 Milano Italy

1979 July 22-27 The Fifth British Academic Conference in Otolaryngology will be held at the University of Birmingham UK Addr V Hammond FRCS., 55 Harley Street, London W 1